US007070974B1

(12) **United States Patent**
Desgroseillers et al.

(10) **Patent No.: US 7,070,974 B1**
(45) **Date of Patent: Jul. 4, 2006**

(54) **METALLOPROTEASES OF THE NEPRILYSIN FAMILY**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/913,329**

(22) PCT Filed: **Feb. 11, 2000**

(86) PCT No.: **PCT/CA00/00147**

§ 371 (c)(1),
(2), (4) Date: **Aug. 21, 2001**

(87) PCT Pub. No.: **WO00/47750**

PCT Pub. Date: **Aug. 17, 2000**

(30) **Foreign Application Priority Data**

Feb. 11, 1999 (CA) 2260376

(51) **Int. Cl.**

C12N 9/48	(2006.01)
C12N 1/20	(2006.01)
C12N 15/00	(2006.01)
C12Q 1/37	(2006.01)
C07H 21/04	(2006.01)

(52) **U.S. Cl.** **435/212**; 435/4; 435/6; 435/252.3; 435/320.1; 435/69.1; 435/71.1; 435/440; 435/24; 536/23.2; 536/23.5

(58) **Field of Classification Search** 435/183, 435/252.3, 320.1, 4, 71.1, 212, 440, 6, 69.1; 536/23.2, 23.1, 23.5; 530/350
See application file for complete search history.

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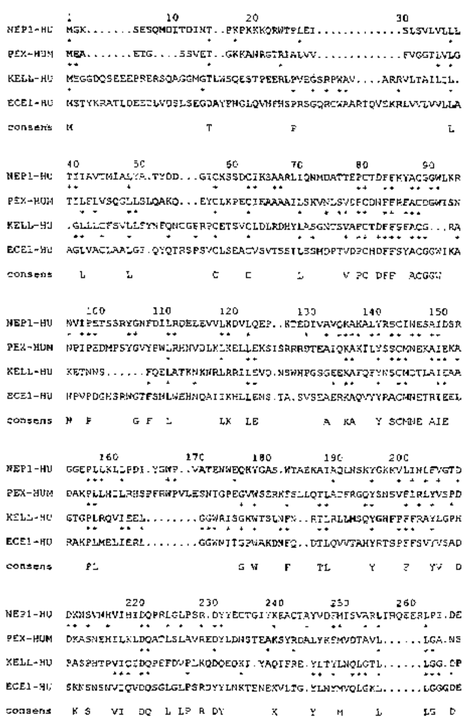
(57) **ABSTRACT**

In this paper, we describe RT-PCR strategies that allowed us to identify and clone members of the NEP-like family. Degenerate oligoncleotide primers corresponding to consensus sequences located on either side of the HEXXH consensus sequence for zincins were designed and used in RT-PCR with mouse and human testis cDNAs. DNA fragments with lengths expected from the sequence of this class of enzympes were obtained. These DNA fragments were cloned and sequenced. Using this PCR strategy and the PCR fragments as probes to screen cDNA libraries, three zincin-like peptidases were identified in addition of known members of the family. The cDNA sequences allowed to derive specific probes for Northern and in situ hybridization, and probe human chromosomes to localize the gene and establish potential links to genetic diseases. Furthermore, these cDNA sequences were used to produce recombinant fusion proteins in *Escherichia coli* in order to raise specific antibodies. Finally, the cDNA sequences were cloned in mammalian expression vectors and transfected in various mammalian cell lines to produce active recombinant enzymes suitable for testing specific inhibitors.

6 Claims, 22 Drawing Sheets



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          1                 10                20                              30
NEP1-HU   MGK.......SESQMDITDINT..PKPKKKQRWTPLEI.............SLSVLVLLL
          *                   *   *           *                               *
PEX-HUM   MEA.......ETG....SSVET..GKKAJRGTRIALVV.............FVGGTLVLG
          **                  *               *                             * *
KELL-HU   MEGGDQSEEEPRERSQAGGMGTLWSQESTPEERLPVEGSRPWAV....ARRVLTAILIL.
          *                   *               * * * **        *             *
ECE1-HU   MSTYKRATLDEEDLVDSLSEGDAYPNGLQVNFHSPRSGQRCWAARTQVEKRLVVLVVLLA

consens   M                   T               P                             L


          40          50                60          70          80          90
NEP1-HU   TIIAVTMIALYA.TYDD...GICKSSDCIKSAARLIQNMDATTEPCTDFFKYACGGWLKR
          **          *                 *     **  **              **  **  **  **
PEX-HUM   TILFLVSQGLLSLQAKQ...EYCLKPECIEAAAAILSKVNLSVDPCDNFFRFACDGWISN
            * *     **              *     *     *  * **  **  **  ***
KELL-HU   .GLLLCFSVLLFYNFQNCGPRPCETSVCLDLRDHYLASGNTSVAPCTDFFSFACG...RA
           **  *   *     *   *   *     *         * *     * **  **** ***     *
ECE1-HU   AGLVACLAALGI.QYQTRSPSVCLSEACVSVTSSILSSMDPTVDPCHDFFSYACGGWIKA

consens     L       L             C     C       L       V PC DFF  ACGGW


            100         110         120         130         140         150
NEP1-HU   NVIPETSSRYGNFDILRDELEVVLKDVLQEP..KTEDIVAVQKAKALYRSCINESAIDSR
          * ***     **    **        **  *           *  * **** **  **  **  **
PEX-HUM   NPIPEDMPSYGVYPWLRHNVDLKLKELLEKSISRRRDTEAIQKAKILYSSCMNEKAIEKA
                        *       *   **                **    * ***   *** *
KELL-HU   KETNNS......FQELATKNKNRLRRILEVQ.NSWHPGSGEEKAFQFYNSCMDTLAIEAA
                      * *   *         **                * **    *   **    **
ECE1-HU   NPVPDGHSRWGTFSNLWEHNQAIIKHLLENS.TA.SVSEAERKAQVYYRACMNETRIEEL

consens   N  P       G F  L        LK  LE           A  KA   Y SCMNE AIE


               160         170         180         190         200
NEP1-HU   GGEPLLKLLPDI.YGWP..VATENWEQKYGAS.WTAEKAIAQLNSKYGKKVLINLFVGTD
             ***  *       **          *                 *     *   * * * * *
PEX-HUM   DAKPLLHILRHSPFRWPVLESNIGPEGVWSERKFSLLQTLATFRGQYSNSVFIRLYVSPD
             **                             * *   *    **      **    * * * *
KELL-HU   GTGPLRQVIEELGGWRISGKWTSLNFN..RTLRLLMSQYGHFPFFRAYLGPH
             **  **  *         ***  *  *  *   **    **        *   ***  *
ECE1-HU   RAKPLMELIERLGGWNITGPWAKDNFQ..DTLQVVTAHYRTSPFFSVYVSAD

consens     PL                        G W    F    TL      Y    F   YV  D


                    220         230         240         250         260
NEP1-HU   DKNSVNHVIHIDQPRLGLPSR.DYYECTGIYKEACTAYVDFMISVARLIRQEERLPI.DE
          **  *   *    **  *  *  *  **        *     *   **   *  *       *
PEX-HUM   DKASNEHILKLDQATLSLAVREDYLDNSTEAKSYRDALYKFMVDTAVL......LGA.NS
                    **        *       *   *                  *     **
KELL-HU   PASPHTPVIQIDQPEFDVPLKQDQEQKI.YAQIFRE.YLTYLNQLGTL......LGG.DP
                 *** **     *       *           ** *  *** *      *** *
ECE1-HU   SKNSNSNVIQVDQSGLGLPSRDYYLNKTENEKVLTG.YLNYMVQLGKL......LGGGDE

consens     K S    VI   DQ   L LP R DY        K     Y   M     L       LG  D
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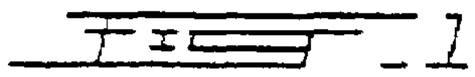

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                270        280        290        300        310        320
NEP1-HU   NQLALEMNKVMELEKEIANATAKPEDRNDPMLLYNKMTLAQIQNNFSLEINGKPFSWLNF
PEX-HUM   SRAEHDMKSVLRLEIKIAEIMIPHENRTSEAMY.NKMNISELSAMIP......QFDWLGY
KELL-HU   SKVQEHSSLSISITSRLFQFLRPLEQRRAQGKLFQMVTIDQLKEMAP......AIDWLSC
ECE1-HU   EAIRPQMQQILDFETALANITIPQEKRRDEELIYHKVTAAELQTLAP......AINWLPF
consens         M      E   A    P E R        K T   L    P          WL

                330        340             350        360        370        380
NEP1-HU   TNEIMSTVNISITNEEDVVVYA....PEYLTKLKPILTKYSARDLQNLMSWRFIMDLVSS
PEX-HUM   IKKVIDTRLYPHLKDISPSENVVVRVPQYFKDLFRILGSERKKTIANYLVWRMVYSRIPN
KELL-HU   LQATFTPMSLSPSQSLVVHDVEYL...KNMSQLVEEMLLKQRDFLQSHMILGLVVTLSPA
ECE1-HU   LNTIFYPVEINESEPIVVYDKEYL...EQISTLINT...TDRCLLNNYMIWNLVRKTSSF
consens                   V                L           L N M W   V

                390             400        410        420        430
NEP1-HU   LSRTYKESR....NAFRKALYGTT.SETATWRRCANYVNGNMENAVGRLYVEAAFAGESK
PEX-HUM   LSRRFQYRW....LEFSRVIQGTT.TLLPQWDKCVNFIESALPYVVGKMFVDVYFQEDKK
KELL-HU   LDSQFQEARRKLSQKLRELTEQPPMPARPRWMKCVEETGTFFEPTLAALFVREAFGPSTR
ECE1-HU   LDQRFQDA....DEKFMEVMYGTKKTCLPRWKFCVSDTENNLGFALGPMFVKATFAEDSK
consens   L    FQ          F      GT      P W  CV             G   FV   F    K

                440        450        460        470        480        490
NEP1-HU   HVVEDLIAQIREVFIQTLD.DLTWMDAETKKRAEEKALAIKERIGYPDDIVSNDNKLNNE
PEX-HUM   EMMEELVEGVRWAFIDMLEKENEWMDAGTKRKAKEKARAVLAKVGYPE.FIMNDTHVNED
KELL-HU   SAAMKLFTAIRDALITRLR.NLPWMNEETQNMAQDKVAQLQVEMGASE.WALKPELARQE
ECE1-HU   SIATEIILEIKKAFEESLS.TLKWMDEETRKSAKEKADAIYNMIGYPN.FIMDPKELDKV
                    (4)  ------------------>
consens         L    IR AFI  L    L WMD ET   A EKA A     GYP
                    (1A/B) ------------------>

                500        510        520        530        540        550
NEP1-HU   YLELNYKEDEYFENIIQNLKFSQSKQLKKLREKVDKDEWISGAAVVNAFYSSGRNQIVFP
PEX-HUM   LKAIKFSEADYFGNVLQTRKYLAQSDFFWLRKAVPKTEWFTNPTTVNAFYSASTNQIRFP
KELL-HU   YND.IQLGSSFLQSVLSCVRSLRARIVQSFLQPHPQHRWKVSPWDVNAYYSVSDHVVVFP
ECE1-HU   FNDYTAVPDLYFENAMRFFNFSWRVTADQLRKAPNRDQWSMTPPMVNAYYSPTKNEIVFP
                                                          (2A/B) ------>
consens           YF N              LR        W    P  VNA YS    N IVFP
```

Fig. 1 (cont'd)

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                560        570        580        590        600        610
NEP1-HU   AGILQPPFFSAQQ.SNSLNYGGIGMVIGHEITHGFDDNGRNFNKDGDLVDWWTQQSASNF
PEX-HUM   AGELQKPFFWGTEYPRSLSYGAIGVIVGHEFTHGFDNNGRKYDKNGNLDPWWSTESEEKF
KELL-HU   AGLLQPPFFHPGY.PRAVNFGAAGSIMAHELLHIFYQL...LLPGGCL.....ACDNHAL
ECE1-HU   AGILQAPFYTRSS.PKALNFGGIGVVVGHELTHAFDDQGREYDKDGNLRPWWKNSSVEAF
consens   AG LQ PFF     P  LN G IG   GHE TH FD  GR   K G L  WW   S   F

                620        630        640        650        660        670
NEP1-HU   KEQSQCMVYQYGNFSWDLAGGQHLNGINTLGENIADNGGLGQAYRAYQNYI..KKNG.EE
PEX-HUM   KEKTKCMINQYSNYYWK.KAGLNVKGKRTLGENIADNGGLREAFRAYRKWINDRRQGLEE
KELL-HU   QEAHLCLKRHYAAF..PLPSRTSFNDSLTFLENAADVGGLAIALQAYSKRL..LRHH.GE
ECE1-HU   KRQTECMVEQYSNY..SVNG.EPVNGRHTLGENIADNGGLKAAYRAYQNWV..KKNG.AE
                                                  (3)
consens   KE   CM  QY N           NG  TLGENIADNGGL  A RAY         G  E

                680        690        700        710        720        730
NEP1-HU   KLLPGLDLNHKQLFFLNFAQVWCGTYRPEYAVNSIKTDVHSPGNFRIIGTLQNSAEFSEA
PEX-HUM   PLLPGITFTNNQLFFLSYAHVRCNSYRPEAAREQVQIGAHSPPQFRVNGAISNFEEFQKA
KELL-HU   TVLPSLDLSPQQIFFRSYAQVMCRKPSPQDSH.....DTHSPPHLRVHGPLSSTPAFARY
ECE1-HU   HSLPTLGLTNNQLFFLGFAQVWCSVRTPESSHEGLITDPHSPSRFRVIGSLSNSKEFSEH
consens     LP L L   QLFFL  AQV C    PE        D HSP  FRV G LSN  EF

                740        750
NEP1-HU   FHCRKNSYMNPEKK.CRVW
PEX-HUM   FNCPPNSTMNRGMDSCRLW
KELL-HU   FRCARGALLNPSSR.CQLW
ECE1-HU   FRCPPGSPMNPPHK.CEVW
                              (5)
consens   F C    S MNP     C  W
```

Fig. 1 (cont'd)

PRIMER	SEQUENCE
(1A)	5'-TGGATGGAT/CGA/CIGG/AIACIA/CA-3'
(1B)	5'-TGGATGGAT/CGA/CIGG/AIACIA/CG-3'
(2A)	5'-A/GTIGTITTT/CCCIGCIGGIA/GT/AIC/TTA/TCA-3'
(2B)	5'-A/GTIGTITTTT/CCCIGCIGGIA/GT/AIC/TTG/CCA-3'
(3)	5'-AIICCICCIA/TC/TA/GTCIGCIG/AC/TA/GTTT/CTC-3'
(4)	5'-GAT/CAAT/CT/CTIGAT/CGAA/GT/CTIAAT/CTGGATGG-3'
(5)	5'-T/CT/CACCAIATICT/GA/GCATCG/TT/CTTCATIGGG/ATG-3'

Fig. 2

Sequence of NL-1 cDNA from mouse

```
                                                                                                                 cgggagccagcaccgtgtgacctcacacccagctcag
ctgctctactccacccggagcccaccttggccagctcaccccaactctgagacatcccaacctagcctttaaggacttgcctagcagtgactgagagcaccagggtcccctgggcacttggggcacagcttacagcattgagagcag
agaccaggacagtgcaccagcttcagtgtgtcctaggcatccgatccgggctccagctgcctctccctagccctggcctgggggcttagcggtgtgccttccacccagaaccggctgatagggaaagtctgagagcccagtgggg

                                                                                                          30
    met val glu arg ala gly trp cys arg lys lys ser pro gly phe val glu tyr gly leu met val leu leu leu leu leu leu gly ala
  1 ATG GTG GAG AGA GCA GGC TGG TGT CGG AAG AAG TCC CCA GGC TTC GTG GAG TAT GGG CTG ATG GTG CTG CTG CTG CTG TTG CTG GGA GCC
                                                                                                          60
    ile val thr leu gly val phe tyr ser ile gly lys gln leu pro leu leu thr ser leu leu his phe ser trp asp glu arg thr val
 91 ATA GTG ACT CTG GGT GTC TTC TAC AGC ATA GGG AAG CAG CTG CCC CTC TTA ACT AGC CTG CTA CAC TTC TCC TGG GAT GAG AGG ACG GTT
                                                                                                          90
    val lys arg ala leu arg asp ser ser leu lys ser asp ile cys thr thr pro ser cys val ile ala ala ala arg ile leu glu asn
181 GTA AAA CGA GCC CTC AGG GAT TCA TCA CTG AAA AGT GAC ATC TGC ACC ACC CCA AGC TGT GTG ATA GCA GCT GCC AGA ATC CTC GAA AAC
                                                                                                         120
    met asp gln ser arg asn pro cys glu asn phe tyr gln tyr ala cys gly gly trp leu arg his his val ile pro glu thr asn ser
271 ATG GAC CAA TCG AGG AAC CCC TGT GAA AAC TTC TAC CAG TAC GCC TGC GGA GGC TGG CTG AGG CAC CAC GTG ATC CCA GAG ACC AAC TCC
                                                                                                         150
    arg tyr ser val phe asp ile leu arg asp glu leu glu val ile leu lys gly val leu glu asp ser thr ser gln his arg pro ala
361 CGA TAC AGC GTC TTT GAC ATC CTG CGG GAC GAG CTG GAG GTT ATC CTC AAA GGG GTG CTG GAG GAT TCC ACT TCC CAG CAT CGC CCG GCC
                                                                                                         180
    val glu lys ala lys thr leu tyr arg ser cys met asn gln ser val ile glu lys arg asp ser glu pro leu leu ser val leu lys
451 GTG GAG AAG GCC AAG ACA CTA TAT CGC TCC TGC ATG AAC CAA AGT GTG ATC GAG AAG AGA GAC TCT GAG CCC CTG CTG AGC GTC TTA AAA
                                                                                                         210
    met val gly gly trp pro val ala met asp lys trp asn glu thr met gly leu lys trp glu leu glu arg gln leu ala val leu asn
541 ATG GTA GGA GGT TGG CCT GTG GCC ATG GAT AAG TGG AAC GAG ACC ATG GGC CTC AAG TGG GAA CTG GAG CGA CAG TTG GCT GTG TTG AAC
                                                                                                         240
    ser gln phe asn arg arg val leu ile asp leu phe ile trp asn asp asp gln asn ser ser arg his val ile tyr ile asp gln pro
631 TCG CAG TTC AAC AGG CGG GTC CTC ATC GAC CTC TTC ATC TGG AAT GAC GAC CAG AAC TCC AGC CGG CAT GTC ATC TAC ATA GAC CAG CCC
                                                                                                         270
    thr leu gly met pro ser arg glu tyr tyr phe gln glu asp asn asn his lys val arg lys ala tyr leu glu phe met thr ser val
721 ACC TTG GGC ATG CCA TCC CGG GAG TAC TAT TTC CAG GAG GAC AAC AAC CAC AAG GTA CGG AAA GCC TAC CTG GAG TTC ATG ACG TCA GTG
```

FIG. 3

```
                                                                                                                                300
    ala thr met leu arg lys asp gln asn leu ser lys glu ser ala met val arg glu glu met ala glu val leu glu leu glu thr his
811 GCC ACT ATG CTT AGG AAA GAC CAG AAC CTG TCC AAG GAG AGC GCC ATG GTG CGG GAG GAG ATG GCG GAG GTG CTG GAA CTG GAG ACG CAT
                                                                                                                                330
    leu ala asn ala thr val pro gln glu lys arg his asp val thr ala leu tyr his arg met asp leu met glu leu gln glu arg phe
901 CTG GCC AAC GCC ACA GTC CCC CAG GAG AAA AGG CAT GAT GTC ACT GCC CTG TAC CAC CGA ATG GAC CTG ATG GAG CTA CAG GAA AGG TTT
                                                                                                                                360
    gly leu lys gly phe asn trp thr leu phe ile gln asn val leu ser ser val glu val glu leu phe pro asp glu glu val val val
991 GGT CTG AAG GGG TTT AAC TGG ACT CTC TTC ATA CAA AAC GTG TTG TCT TCT GTG GAA GTC GAG CTG TTC CCA GAT GAG GAG GTG GTG GTC
                                                                                                                                390
    tyr gly ile pro tyr leu glu asn leu glu asp ile ile asp ser tyr ser ala arg thr met gln asn tyr leu val trp arg leu val
1081 TAC GGC ATC CCC TAC CTG GAG AAT CTG GAG GAT ATC ATT GAT AGC TAC TCA GCA CGG ACC ATG CAG AAC TAC CTG GTA TGG CGC CTG GTG
                                                                                                                                420
    leu asp arg ile gly ser leu ser gln arg phe lys glu ala arg val asp tyr arg lys ala leu tyr gly thr thr val glu glu val
1171 CTA GAT CGA ATT GGC AGC CTG AGC CAG AGA TTC AAA GAG GCG CGT GTG GAC TAC CGC AAG GCG CTG TAC GGC ACG ACC GTG GAG GAG GTA
                                                                                                                                450
    arg trp arg glu cys val ser tyr val asn ser asn met glu ser ala val gly ser leu tyr ile lys arg ala phe ser lys asp ser
1261 CGC TGG CGA GAG TGT GTC AGC TAT GTC AAC AGT AAC ATG GAG AGC GCC GTG GGC TCC CTC TAC ATC AAG CGG GCC TTC TCC AAG GAC AGC
                                                                                                                                480
    lys ser thr val arg glu leu ile glu lys ile arg ser val phe val asp asn leu asp glu leu asn trp met asp glu glu ser lys
1351 AAG AGC ACG GTC AGA GAG CTG ATT GAG AAG ATA AGG TCC GTG TTT GTG GAT AAC CTG GAT GAG CTG AAC TGG ATG GAC GAG GAA TCC AAG
                                                                                                                                510
    lys lys ala gln glu lys ala met asn ile arg glu gln ile gly tyr pro asp tyr ile leu glu asp asn asn lys his leu asp glu
1441 AAG AAG GCC CAG GAA AAG GCC ATG AAT ATA CGG GAA CAG ATT GGC TAC CCT GAC TAC ATT TTG GAA GAT AAC AAT AAA CAC CTG GAT GAG
                                                                                                                                540
    glu tyr ser ser leu thr phe tyr glu asp leu tyr phe glu asn gly leu gln asn leu lys asn asn ala gln arg ser leu lys lys
1531 GAA TAC TCC AGT TTG ACT TTC TAT GAG GAC CTG TAT TTT GAG AAC GGA CTT CAG AAC CTC AAG AAC AAT GCC CAG AGG AGC CTC AAG AAG
                                                                                                                                570
    leu arg glu lys val asp gln asn leu trp ile ile gly ala ala val val asn ala phe tyr ser pro asn arg asn gln ile val phe
1621 CTT CGG GAA AAG GTG GAC CAG AAT CTC TGG ATC ATC GGG GCT GCA GTG GTC AAT GCA TTC TAC TCC CCA AAC AGA AAC CAG ATC GTC TTT
```

Fig. 3 (cont'd)

```
                                                                                                                600
     pro ala gly ile leu gln pro pro phe phe ser lys asp gln pro gln ser leu asn phe gly gly ile gly met val ile gly his glu
1711 CCA GCA GGG ATT CTC CAG CCG CCC TTC TTC AGC AAG GAC CAA CCA CAG TCC TTG AAT TTT GGG GGC ATC GGG ATG GTG ATT GGG CAC GAG
                                                                                                                630
     ile thr his gly phe asp asp asn gly arg asn phe asp lys asn gly asn met leu asp trp trp ser asn phe ser ala arg his phe
1801 ATC ACA CAC GGC TTT GAT GAT AAT GGT CGT AAC TTT GAC AAG AAC GGC AAC ATG CTG GAC TGG TGG AGT AAC TTC TCG GCC CGG CAC TTC
                                                                                                                660
     gln gln gln ser gln cys met ile tyr gln tyr gly asn phe ser trp glu leu ala asp asn gln asn val asn gly phe ser thr leu
1891 CAA CAG CAG TCG CAA TGC ATG ATC TAT CAG TAC GGC AAC TTC TCT TGG GAA CTA GCA GAC AAC CAG AAT GTG AAC GGA TTC AGT ACC CTC
                                                                                                                690
     gly glu asn ile ala asp asn gly gly val] arg gln ala tyr lys ala tyr leu arg trp leu ala asp gly gly lys asp gln arg leu
1981 GGG GAG AAC ATT GCC GAC AAC GGA GGT GTG] CGA CAG GCA TAC AAG GCT TAC CTA CGG TGG CTG GCT GAT GGC GGC AAA GAT CAG CGA CTG
                                                                                                                720
     pro gly leu asn leu thr tyr ala gln leu phe phe ile asn tyr ala gln val trp cys gly ser tyr arg pro glu phe ala val gln
2071 CCG GGA CTG AAC CTG ACC TAT GCC CAG CTT TTC TTC ATC AAC TAT GCC CAG GTG TGG TGT GGG TCC TAT AGG CCG GAG TTC GCC GTC CAG
                                                                                                                750
     ser ile lys thr asp val his ser pro leu lys tyr arg val leu gly ser leu gln asn leu pro gly phe ser glu ala phe his cys
2161 TCC ATC AAG ACG GAC GTC CAC AGT CCT CTT AAG TAC AGG GTG CTG GGC TCA CTA CAG AAC CTG CCA GGC TTC TCT GAG GCA TTC CAC TGC
                                                     765
     pro arg gly ser pro met his pro met lys arg cys arg ile trp ***
2251 CCA CGA GGC AGC CCC ATG CAC CCC ATG AAG CGA TGT CGC ATC TGG TAG CCAAGGCTGAGCTATGCTGCGGCCCACGCCCCGCCACCCAGAGGCTTCGCGAATG

2354 GTGTAGCTGGCAGAGATGTGCAGGTCTTTGCCTGAAGGCCACCGGAGCCACCAGCCAGCCCTCCGCGCCCAGCCTAGAGTGTAGCCACCCGCCCACACCCGGGATGAGTGGTGCCGGTC

2473 CTGCGCCCCCTCAGGCCAGTGAGGGTCAGCAGCCCAGGAAGAGCAGTCAGCTGCCTTCCACCCTCTCCATAGTGTGTGGCTAAATGTTCTCGAGCTTCAGACTTGAGCTAAGTAAACGC

2925 TTC
```

FIG. 3 (cont'd)

Sequence of NL-2 cDNA from humans

```
                                                                                                                                  28
                met val glu ser ala gly arg ala gly gln lys arg pro gly phe leu glu gly gly leu leu leu leu leu leu leu val thr
  1 GTG GGG ATG GTG GAG AGC GCC GGC CGT GCA GGG CAG AAG CGC CCG GGG TTC CTG GAG GGG GGG CTG CTG CTG CTG CTG CTG CTG GTG ACC
                                                                                                                                  58
    ala ala leu val ala leu gly val leu tyr ala asp arg arg gly lys gln leu pro arg leu ala ser arg leu cys phe leu gln glu
 91 GCT GCC CTG GTG GCC TTG GGT GTC CTC TAC GCC GAC CGC AGA GGG AAG CAG CTG CCA CGC CTT GCT AGC CGG CTG TGC TTC TTA CAG GAG
                                                                                                                                  88
    glu arg thr phe val lys arg lys pro arg gly ile pro glu ala gln glu val ser glu val cys thr thr pro gly cys val ile ala
181 GAG AGG ACC TTT GTA AAA CGA AAA CCC CGA GGG ATC CCA GAG GCC CAA GAG GTG AGC GAG GTC TGC ACC ACC CCT GGC TGC GTG ATA GCA
                                                                                                                                 118
    ala ala arg ile leu gln asn met asp pro thr thr glu pro cys asp asp phe tyr gln phe ala cys gly gly trp leu arg arg his
271 GCC GCC AGG ATC CTC CAG AAC ATG GAC CCG ACC ACG GAA CCG TGT GAC GAC TTC TAC CAG TTT GCA TGC GGA GGC TGG CTG CGG CGC CAC
                                                                                                                                 148
    val ile pro glu thr asn ser arg tyr ser ile phe asp val leu arg asp glu leu glu val ile leu lys ala val leu glu asn ser
361 GTG ATC CCT GAG ACC AAC TCA AGA TAC AGC ATC TTT GAC GTC CTC CGC GAC GAG CTG GAG GTC ATC CTC AAA GCG GTG CTG GAG AAT TCG
                                                                                                                                 178
    thr ala lys asp arg pro ala val glu lys ala arg thr leu tyr arg ser cys met asn gln ser val ile glu lys arg gly ser gln
451 ACT GCC AAG GAC CGG CCG GCT GTG GAG AAG GCC AGG ACG CTG TAC CGC TCC TGC ATG AAC CAG AGT GTG ATA GAG AAG CGA GGC TCT CAG
                                                                                                                                 208
    pro leu leu asp ile leu glu val val gly gly trp pro val ala met asp arg trp asn glu thr val gly leu glu trp glu leu glu
541 CCC CTG CTG GAC ATC TTG GAG GTG GTG GGA GGC TGG CCG GTG GCG ATG GAC AGG TGG AAC GAG ACC GTA GGA CTC GAG TGG GAG CTG GAG
                                                                                                                                 238
    arg gln leu ala leu met asn ser gln phe asn arg arg val leu ile asp leu phe ile trp asn asp asp gln asn ser ser arg his
631 CGG CAG CTG GCG CTG ATG AAC TCA CAG TTC AAC AGG CGC GTC CTC ATC GAC CTC TTC ATC TGG AAC GAC GAC CAG AAC TCC AGC CGG CAC
                                                                                                                                 268
    ile ile tyr ile asp gln pro thr leu gly met pro ser arg glu tyr tyr phe asn gly gly ser asn arg lys val arg glu ala tyr
721 ATC ATC TAC ATA GAC CAG CCC ACC TTG GGC ATG CCC TCC CGA GAG TAC TAC TTC AAC GGC GGC AGC AAC CGG AAG GTG CGG GAA GCC TAC
                                                                                                                                 298
    leu gln phe met val ser val ala thr leu leu arg glu asp ala asn leu pro arg asp ser cys leu val gln glu asp met val gln
811 CTG CAG TTC ATG GTG TCA GTG GCC ACG TTG CTG CGG GAG GAT GCA AAC CTG CCC AGG GAC AGC TGC CTG GTG CAG GAG GAC ATG GTG CAG
                                                                                                                                 328
```

FIG. 4

Fig. 4 (cont'd)

```
val leu glu leu glu thr gln leu ala lys ala thr val pro gln glu glu arg his asp val ile ala leu tyr his arg met gly leu
901 GTT CTG GAG CTG GAG ACA CAG CTG GCC AAG GCC ACG GTA CCC CAG GAG GAG AGA CAC GAC GTC ATC GCC TTG TAC CAC CGG ATG GGA CTG
                                                                                                                    358
glu glu leu ser gln phe gly leu lys gly phe asn trp thr leu phe glu ile val leu ser ser val lys ile lys leu leu
991 GAG GAG CTG CAA AGC CAG TTT GGC CTG AAG GCA TTT AAC TGG ACT CTG TTC ATA CAA ACT GTG CTA TCC TCT GTC AAA ATC AAG CTG CTG
                                                                                                                    388
pro asp glu glu val val tyr gly ile pro tyr leu gln asn leu gln asn ile ile asp thr tyr ser ala arg thr ile gln asn
1081 CCA GAT GAG GAA GTG GTG GTC TAT GGC ATC CCC TAC CTG CAG AAC ATC ATC GAC AAC CTT GAA AAC ATC ATC GAC ACC TAC TCA GCC AGG ACC ATA CAG AAC
                                                                                                                    418
tyr leu val trp arg leu val leu asp arg ile gly ser leu ser gln arg phe lys asp thr arg val asn tyr arg lys ala leu phe
1171 TAC CTG GTC TGG CGC CTG GTG CTG GAC CGC ATT GGT AGC CAG AGA TTC AAG GAC ACA CGA GTG AAC TAC CGC AAG GCG CTG TTT
                                                                                                                    448
gly thr met val glu val arg trp arg glu cys val gly tyr val asn ser asn met glu asn ala val gly ser leu tyr val arg
1261 GGC ACA ATG GTG GAG GTG CGC TGG CGT GAA TGT GTG GGC TAC GTC AAC AGC AAC ATG GAG AAC GCC GTG GGC TCC CTC TAC GTC AGG
                                                                                                                    478
glu ala phe pro gly asp ser lys ser met val arg glu leu ile asp lys val arg thr val phe val glu thr leu asp glu leu gly
1351 GAG GCG TTC CCT GGA GAC AAG AGC ATG GTC AGA GAA CTC ATT GAC AAG GTG CGG ACA GTG TTT GTG GAG ACG CTG GAC GAG CTG GGC
                                                                                                                    508
trp met asp glu lys lys ala gln glu lys ala met ser ile arg glu gln ile gly his pro asp tyr ile leu glu glu
1441 TGG ATG GAC GAG AAG AAG GCG CAG GAG AAG GCC ATG AGC ATC CGG GAG CAG ATC GGG CAC CCT GAC TAC ATC CTG GAG GAG
                                                                                                                    538
met asn arg arg leu asp glu glu tyr ser asn leu asn phe ser glu asp leu tyr phe glu asn ser leu gln asn leu lys val gly
1531 ATG AAC AGG CGC CTG GAC GAG TAC TCC AAT CTG AAC TTC TCA GAG GAC CTG TAC TTT GAG AAC AGT CTG CAG AAC CTC AAG GTG GGC
                                                                                                                    568
ala gln arg ser leu arg lys leu asp glu lys val asp pro asn leu trp ile ile gly ala ala val val asn ala phe tyr ser pro
1621 GCC CAG CGG AGC CTC AGG AAG CTT CGG GAA AAG GTG GAC CCA AAT CTC TGG ATC ATC GGG GCG GCG GTG GTC AAT GCG TTC TAC TCC CCA
                                                                                                                    598
asn arg asn gln ile val phe pro ala gly ile leu gln pro phe ser lys glu gln pro gln ala leu asn phe gly gly gly ile
1711 AAC CGA AAC CAG ATT GTA TTC CCT GCC GGG ATC CTC CAG CCC CCC TTC TTC AGC AAG GAG CAG CCA CAG GCC CAG TTG AAC TTT GCA GGC ATT
                                                                                                                    628
```

```
     gly met val ile gly his glu ile thr his gly phe asp asp asn gly arg asn phe asp lys asn gly asn met met asp trp trp ser
1801 GGG ATG GTG ATC GGG CAC GAG ATC ACG CAC GGC TTT GAC GAC AAT GGC CGG AAC TTC GAC AAG AAT GGC AAC ATG ATG GAT TGG TGG AGT
                                                                                                                    658
     asn phe ser thr gln his phe arg glu gln ser glu cys met ile tyr gln tyr gly asn tyr ser trp asp leu ala asp glu gln asn
1891 AAC TTC TCC ACC CAG CAC TTC CGG GAG CAG TCA GAG TGC ATG ATC TAC CAG TAC GGC AAC TAC TCC TGG GAC CTG GCA GAC GAA CAG AAC
                                                                                                                    688
     val asn gly phe asn thr leu gly glu asn ile ala asp asn gly gly val arg gln ala tyr lys ala tyr leu lys trp met ala glu
1981 GTG AAC GGA TTC AAC ACC CTT GGG GAA AAC ATT GCT GAC AAC GGA GGG GTG CGG CAA GCC TAT AAG GCC TAC CTC AAG TGG ATG GCA GAG
                                                                                                                    718
     gly gly lys asp gln gln leu pro gly leu asp leu thr his glu gln leu phe phe ile asn tyr ala gln val trp cys gly ser tyr
2071 GGT GGC AAG GAC CAG CAG CTG CCC GGC CTG GAT CTC ACC CAT GAG CAG CTC TTC TTC ATC AAC TAT GCC CAG GTG TGG TGC GGG TCC TAC
                                                                                                                    748
     arg pro glu phe ala ile gln ser ile lys thr asp val his ser pro leu lys tyr arg val leu gly ser leu gln asn leu ala ala
2161 CGG CCC GAG TTC GCC ATC CAA TCC ATC AAG ACA GAC GTC CAC AGT CCC CTG AAG TAC AGG GTA CTG GGG TCG CTG CAG AAC CTG GCC GCC
                                                                                                                    770
     phe ala asp thr phe his cys ala arg gly thr pro met his pro lys glu arg cys arg val trp ter
2251 TTC GCA GAC ACG TTC CAC TGT GCC CGG GGC ACC CCC ATG CAC CCC AAG GAG CGA TGC CGC GTG TGG TAG CCA AGG CCC TGC CGC GCT GTG

2341 CGG CCC ACG CCC ACC CGC TGC TCG GAG GCA TCT GTG CGA AGG TGC AGC TAG CGG CGA CCC AGT GTA CGT CCC GCC CCG GCC AAC CAT GCC

2431 AAG CCT GCC TGC CAG GCC TCT GCG CCT GGC CTA GGG TGC AGC CAC CTG CCT GAC ACC CAG GGA TGA GCA GTG TCC AGT GCA GTA CCT GGA

2521 CCG GAG CCC CCT TCA CAG ACA CCC GCG GGG CTC AGT GCC CCC GTC ACA ACT CTG TAG AGA CAA TCA ACT GTG TCC TGC CCA CCC TTC AAG

2611 GTG CAT TGT CTT CCA GTA TCT ACA GCT TCA GAA CTT GAG CTA AGT AAA TGC TTT CAA AGA AAA AAA
```

FIG. 4 (cont'd)

Sequence of NL-3 cDNA from human

```
    1 GGC GCT GGG AGA CAC CGG ACG CCC GCT CGG CTG CGC TGC GGC TCA GGC CCC CGC TCG GCC CGA CCC GCT CGG TCA CCG CCG GCT CGG GCG

   91 CGC ACC TGC CGG CTG CGG CCC CAG GGC CAT GCG GAG GCC CAC GAG GAG GCC GGC GGC CAC GCG CAT CCC GTA GCC CAG GTG GCC CAG GTC
                                                                                                                        22
                                    met glu pro pro tyr ser leu thr ala his tyr asp glu phe gln glu val lys tyr val ser arg
  181 TGC ACC GCG GCG GCC TCG GCG CCG ATG GAG CCC CCG TAT TCG CTG ACG GCG CAC TAC GAT GAG TTC CAA GAG GTC AAG TAC GTG AGC CGC
                                                                                                                        52
      cys gly ala gly gly ala arg gly ala ser leu pro pro gly phe pro leu gly ala ala arg ser ala thr gly ala arg ser gly leu
  271 TGC GGC GCG GGG GGC GCG CGC GGG GCC TCC CTG CCC CCG GGC TTC CCG TTG GGC GCT GCG CGC AGC GCC ACC GGG GCC CGG TCC GGG CTG
                                                                                                                        82
      pro arg trp asn arg arg glu val cys leu leu ser gly leu val phe ala ala gly leu cys ala ile leu ala ala met leu ala leu
  361 CCG CGC TGG AAC CGG CGC GAG GTG TGC CTG CTG TCG GGG CTG GTG TTC GCC GCC GGC CTC TGC GCC ATT CTG GCG GCT ATG CTG GCC CTC
                                                                                                                       112
      lys tyr leu gly pro val ala ala gly gly gly ala cys pro glu gly cys pro glu arg lys ala phe ala arg ala ala arg phe leu
  451 AAG TAC CTG GGC CCG GTC GCG GCC GGC GGC GGC GCC TGT CCC GAG GGC TGC CCT GAG CGC AAG GCC TTC GCG CGC GCC GCT CGC TTC CTG
                                                                                                                       142
      ala ala asn leu asp ala ser ile asp pro cys gln asp phe tyr ser phe ala cys gly gly trp leu arg arg his ala ile pro asp
  541 GCC GCC AAC CTG GAC GCC AGC ATC GAC CCA TGC CAG GAC TTC TAC TCG TTC GCC TGC GGC GGT TGG CTG CGG CGC CAC GCC ATC CCC GAC
                                                                                                                       172
      asp lys leu thr tyr gly thr ile ala ala ile gly glu gln asn glu glu arg leu arg arg leu leu ala arg pro gly gly gly pro
  631 GAC AAG CTC ACC TAT GGC ACC ATC GCG GCA ATC GGC GAG CAA AAC GAG GAG CGC CTA CGG CGC CTG CTG GCG CGG CCC GGG GGT GGG CCT
                                                                                                                       202
      gly gly ala ala gln arg lys val arg ala phe phe arg ser cys leu asp met arg glu ile glu arg leu gly pro arg pro met leu
  721 GGC GGC GCG GCC CAG CGC AAG GTG CGC GCC TTC TTC CGC TCG TGC CTC GAC ATG CGC GAG ATC GAG CGA CTG GGC CCG CGA CCC ATG CTA
                                                                                                                       232
      glu val ile glu asp cys gly gly trp asp leu gly gly ala glu glu arg pro gly val ala ala arg trp asp leu asn arg leu leu
  811 GAG GTC ATC GAG GAC TGC GGG GGC TGG GAC CTG GGC GGC GCG GAG GAG CGT CCG GGG GTC GCG GCG CGA TGG GAC CTC AAC CGG CTG CTG
                                                                                                                       262
      tyr lys ala gln gly val tyr ser ala ala ala leu phe ser leu thr val ser leu asp asp arg asn ser ser arg tyr val ile arg
  901 TAC AAG GCG CAG GGC GTG TAC AGC GCC GCC GCG CTC TTC TCG CTC ACG GTC AGC CTG GAC GAC AGG AAC TCC TCG CGC TAC GTC ATC CGC
                                                                                                                       292
      ile asp gln asp gly leu thr leu pro glu arg thr leu tyr leu ala gln asp glu asp ser glu lys val leu ala ala tyr arg val
  991 ATT GAC CAG GAT GGG CTC ACC CTG CCA GAG AGG ACC CTG TAC CTC GCT CAG GAT GAG GAC AGT GAG AAG GTC CTG GCA GCA TAC AGG GTG
                                                                                                                       322
      phe met glu arg val leu ser leu leu gly ala asp ala val glu gln lys ala gln glu ile leu gln val glu gln gln leu ala asn
 1081 TTC ATG GAG CGA GTG CTC AGC CTC CTG GGT GCA GAC GCT GTG GAA CAG AAG GCC CAA GAG ATC CTG CAA GTG GAG CAG CAG CTG GCC AAC
```

FIG_5

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                                                                                                                                352
     ile thr val ser glu tyr asp asp leu arg arg asp val ser ser met tyr asn lys val thr leu gly gln leu gln lys ile thr pro
1171 ATC ACT GTG TCA GAG TAT GAC GAC CTA CGG CGA GAT GTC AGC TCC ATG TAC AAC AAG GTG ACG CTG GGG CAG CTG CAG AAG ATC ACC CCC
                                                                                                                                382
     his leu arg trp lys trp leu leu asp gln ile phe gln glu asp phe ser glu glu glu glu val val leu leu ala thr asp tyr met
1261 CAC TTG CGG TGG AAG TGG CTG CTA GAC CAG ATC TTC CAG GAG GAC TTC TCA GAG GAA GAG GAG GTG GTG CTG CTG GCG ACA GAC TAC ATG
                                                                                                                                412
     gln gln val ser gln leu ile arg ser thr pro his arg val leu his asn tyr leu val trp arg val val val val leu ser glu his
1351 CAG CAG GTG TCG CAG CTC ATC CGC TCC ACA CCC CAC CGG GTC CTG CAC AAC TAC CTG GTG TGG CGC GTG GTG GTG GTC CTG AGT GAA CAC
                                                                                                                                442
     leu ser pro pro phe arg glu ala leu his glu leu ala gln glu met glu gly ser asp lys pro gln glu leu ala arg val cys leu
1441 CTG TCC CCG CCA TTC CGT GAG GCA CTG CAC GAG CTG GCA CAG GAG ATG GAG GGC AGC GAC AAG CCA CAG GAG CTG GCC CGG GTC TGC TTG
                                                                                                                                472
     gly gln ala asn arg his phe gly met ala leu gly ala leu phe val his glu his phe ser ala ala ser lys ala lys val gln gln
1531 GGC CAG GCC AAT CGC CAC TTT GGC ATG GCG CTT GGC GCC CTC TTT GTA CAT GAG CAC TTC TCA GCT GCC AGC AAA GCC AAG GTG CAG CAG
                                                                                                                                502
     leu val glu asp ile lys tyr ile leu gly gln arg leu glu glu leu asp trp met asp ala glu thr arg ala ala ala arg ala lys
1621 CTA GTG GAA GAC ATC AAG TAC ATC CTG GGC CAG CGC CTG GAG GAG CTG GAC TGG ATG GAC GCC GAG ACC AGG GCT GCT GCT CGG GCC AAG
                                                                                                                                532
     leu gln tyr met met val met val gly tyr pro asp phe leu leu lys pro asp ala val asp lys glu tyr glu phe glu val his glu
1711 CTC CAG TAC ATG ATG GTG ATG GTC GGC TAC CCG GAC TTC CTG CTG AAA CCC GAT GCT GTG GAC AAG GAG TAT GAG TTT GAG GTC CAT GAG
                                                                                                                                562
     lys thr tyr phe lys asn ile leu asn ser ile arg phe ser ile gln leu ser val lys lys ile arg gln glu val asp lys ser thr
1801 AAG ACC TAC TTC AAG AAC ATC TTG AAC AGC ATC CGC TTC AGC ATC CAG CTC TCA GTT AAG AAG ATT CGG CAG GAG GTG GAC AAG TCC ACG
                                                                                                                                592
     trp leu leu pro pro gln ala leu asn ala tyr tyr leu pro asn lys asn gln met val phe pro ala gly ile leu gln pro thr leu
1891 TGG CTG CTC CCC CCA CAG GCG CTC AAT GCC TAC TAT CTA CCC AAC AAG AAC CAG ATG GTG TTC CCC GCG GGC ATC CTG CAG CCC ACC CTG
                                                                                                                                622
     tyr asp pro asp phe pro gln ser leu asn tyr gly gly ile gly thr ile ile gly his glu leu thr his gly tyr asp asp trp gly
1981 TAC GAC CCT GAC TTC CCA CAG TCT CTC AAC TAC GGG GGC ATC GGC ACC ATC ATT GGA CAT GAG CTG ACC CAC GGC TAC GAC GAC TGG GGG
                                                                                                                                652
```

Fig. 5 (cont'd)

```
     gly gln tyr asp arg ser gly asn leu leu his trp trp thr glu ala ser tyr ser arg phe leu arg lys ala glu cys ile val arg
2071 GGC CAG TAT GAC CGC TCA GGG AAC CTG CTG CAC TGG TGG ACG GAG GCC TCC TAC AGC CGC TTC CTG CGA AAG GCT GAG TGC ATC GTC CGT
                                                                                                                    682
     leu tyr asp asn phe thr val tyr asn gln arg val asn gly lys his thr leu gly glu asn ile ala asp met gly gly leu lys leu
2161 CTC TAT GAC AAC TTC ACT GTC TAC AAC CAG CGG GTG AAC GGG AAA CAC ACG CTT GGG GAG AAC ATC GCA GAT ATG GGC GGC CTC AAG CTG
                                                                                                                    712
     ala tyr his ala tyr gln lys trp val arg glu his gly pro glu his pro leu pro arg leu lys tyr thr his asp gln leu phe phe
2251 GCC TAC CAC GCC TAT CAG AAG TGG GTG CGG GAG CAC GGC CCA GAG CAC CCA CTT CCC CGG CTC AAG TAC ACA CAT GAC CAG CTC TTC TTC
                                                                                                                    742
     ile ala phe ala gln asn trp cys ile lys arg arg ser gln ser ile tyr leu gln val leu thr asp lys his ala pro glu his tyr
2341 ATT GCC TTT GCC CAG AAC TGG TGC ATC AAG CGG CGG TCG CAG TCC ATC TAC CTG CAG GTG CTG ACT GAC AAG CAT GCC CCT GAG CAC TAC
                                                                                                                    772
     arg val leu gly ser val ser gln phe glu glu phe gly arg val leu his cys pro lys val ser pro met asn pro ala his lys cys
2431 AGG GTG CTG GGC AGT GTG TCC CAG TTT GAG GAG TTT GGC CGG GTT TTA CAC TGT CCA AAG GTC TCA CCC ATG AAC CCT GCC CAC AAG TGT
                775
     ser val trp ter
2521 TCC GTG TGG TGA CCC TGG CTG CCC GCC TGC ACG CCC CCA CTG CCC CCG CAC GAA TCA CCT CCT GCT GGC TAC CGG GGC AGG CAT GCA CCC

2611 GGT GCC AGC CCC GCT CTG GGC ACC ACC TGC CTT CCA GCC CCT CCA GGA CCC GGT CCC CCT GCT GCC CCT CAC TTC AGG AGG GGC CTG GAG

2701 CAG GGT GAG GCT GGA CTT TGG GGG GCT GTG AGG GAA ATA TAC TGG GGT CCC CAG ATT CTG CTC TAA GGG GGC CAG ACC CTC TGC CAG GCT

2791 GGA TTG TAC GGG CCC CAC CTT CGC TGT GTT CTT GCT GCA AGT CTG GTC AAA TAA ATC ACT GCA CTG TTA AAA AAA AAA AAA
```

Fig. 5 (cont'd)

Sequence comparison between NEP, NL1, NL2 and NL3

```
          1                   10          20          30          40
NEP-HUM   MG...........KSESQMDITDINTPKPKKKQRWTPLEISLSVLVLL..LTII...AV
          *                                 *       *       *
NL1-MOU   MV...........ERAGWCRKKSPGFVEYGLMVLLLLLLGAIVTLG.V..FYSI.GKQL
          **              * **    * *** * **  ****   * * **          ****
NL2-HUM   MV...........ESAGRAGQKRPGFLEGGLLLLLLLVTAALVALGVL..YADRRGKQL
          *                         *   *   *       **  * *
NL3-HUM   MEPPYSLTAHYDEFQEVKYVSRCGAGGARGASLPPGFPLGAARSATGARSGLPRWNRREV

                    50                                  60          70
NEP-HUM   TMIA.......LYATYDD.................GICKSSDCIKSAARLIQ.NMDATT
                      *   *                       **    *   ***    ***
NL1-MOU   PLLTSL.....LHFSWDERTVVKR...ALRDSSLKSDICTTPSCVIAAARILE.NMDQSR
          * * *       * *   *** ***             *  **** *********  ***
NL2-HUM   PRLASR.....LCFLQEERTFVKRKPRGIPEAQEVSEVCTTPGCVIAAARILQ.NMDPTT
            *         **          *               * *        ***- *  * *
NL3-HUM   CLLSGLVFAAGLCAILAAMLALKYLGPVAAGGGACPEGCPERKAFARAARFLAANLDASI

           80          90          100         110         120         130
NEP-HUM   EPCTDFFKYACGGWLKRNVIPETSSRYGNFDILRDELEVVLKDVLQEPKTEDIVAVQ.KA
           **   *  *******    ***** ***  ********** ** **          **  **
NL1-MOU   NPCENFYQYACGGWLRHHVIPETNSRYSVFDILRDELEVILKGVLEDSTSQHRPAVE.KA
           **   *** ******* ********** ** ********** *** **    ***** **
NL2-HUM   EPCDDFYQFACGGWLRRHVIPETNSRYSIFDVLRDELEVILKAVLENSTAKDRPAVE.KA
           ** *** ********** **      *             *  *   *          *   *
NL3-HUM   DPCQDFYSFACGGWLRRHAIPDDKLTYGTIAAIGEQNEERLRRLLARPGGGPGGAAQRKV

           140         150         160         170         180         190
NEP-HUM   KALYRSCINESAIDSRGGEPLLKLLPDIYGWPVATENWEQKYGASWTAEKAIAQLNSKYG
          * ***** * * *  *  **** *    ***** *    *  * *  *  * ***
NL1-MOU   KTLYRSCMNQSVIEKRDSEPLLSVLKMVGGWPVAMDKWNETMGLKWELERQLAVLNSQFN
          ***************** * ***  *  ******** **** ** ********  ******
NL2-HUM   RTLYRSCMNQSVIEKRGSQPLLDILEVVGGWPVAMDRWNETVGLEWELERQLALMNSQFN
          *   ***      **  *  *  *   *  ***           *   * * * * *
NL3-HUM   RAFFRSCLDMREIERLGPRPMLEVIEDCGGWDLGGAEERPGVAARWDLNRLLYKAQGVYS

           200         210         220         230         240         250
NEP-HUM   KKVLINLFVGTDDKNSVNHVIHIDQPRLGLPSRDYYECTGIYKEACTAYVDFMISVARLI
            *** **    ** **  *** **** ** *** **           **  ** ** ***
NL1-MOU   RRVLIDLFIWNDDQNSSRHVIYIDQPTLGMPSREYYFQEDNNHKVRKAYLEFMTSVATML
          ********************* ***************     * *** *** ** **** *
NL2-HUM   RRVLIDLFIWNDDQNSSRHIIYIDQPTLGMPSREYYFNGGSNRKVREAYLQFMVSVATLL
            *   *    ** ****  * *** *  * * *  *                  *     *
NL3-HUM   AAALFSLTVSLDDRNSSRYVIRIDQDGLTLPERTLYLAQDEDSE..KVLAAYRVFMERVL
```

FIG_6

```
                260        270        280        290        300        310
NEP-HUM   RQEERLPIDENQLALEMNKVMELEKEIANATAKP..EDRNDPMLLYNKMTLAQIQNNFSL
          *   *          **  * ***  ****      * * *  **  * *  *  * *
NL1-MOU   RKDQNLSKESAMVREEMAEVLELETHLANATVPQ..EKRHDVTALYHRMDLMELQERFGL
          * * **  * * * * * ******* ** ******  * **** ******* * ***  ***
NL2-HUM   REDANLPRDSCLVQEDMVQVLELETQLAKATVPQ..EERHDVIALYHRMGLEELQSQFGL
            *  *            *  * *** **       * **  *    *  **
NL3-HUM   SL...LGADAV..EQKAQEILQVEQQLANITVSEYDDLRRDVSSMYNKVTLGQLQKITP.

                320        330        340        350        360        370
NEP-HUM   EINGKPFSWLNFTNEIMSTVNISITNEEDVVVYAPEYLTKLKPILTKYSARDLQNLMSWR
            * * *  *     * *       * **** **  *  *  **** **   **
NL1-MOU   ....KGFNWTLFIQNVLSSVEVELFPDEEVVVYGIPYLENLEDIIDSYSARTMQNYLVWR
          ********** *****  * ************ *** *** ***** *******
NL2-HUM   ....KGFNWTLFIQTVLSSVKIKLLPDEEVVVYGIPYLQNLENIIDTYSARTIQNYLVWR
              *                 ****    * *    *    *  ******
NL3-HUM   .....HLRWKWLLDQIF...QEDFSEEEEVVLLATDYMQQVSQLIRSTPHRVLHNYLVWR

                380        390        400        410        420        430
NEP-HUM   FIMDLVSSLSRTYKESRNAFRKALYGTTSETATWRRCANYVNGNMENAVGRLYVEAAFAG
            *  ***  ** *  ******** *  ** *  *** *** *** **   **
NL1-MOU   LVLDRIGSLSQRFKEARVDYRKALYGTTVEEVRWRECVSYVNSNMESAVGSLYIKRAFSK
          ************** ** ***** ** ********** ******* ******   **
NL2-HUM   LVLDRIGSLSQRFKDTRVNYRKALFGTMVEEVRWRECVGYVNSNMENAVGSLYVREAFPG
            *    **  *              *       * * *  *     * * * * * *
NL3-HUM   VVVVLSEHLSPPFREALHELAQEMEGSDKPQELARVCLGQANRHFGMALGALFVHEHFSA

                440        450        460        470        480        490
NEP-HUM   ESKHVVEDLIAQIREVFIQTLDDLTWMDAETKKRAEEKALAIKERIGYPDDIVSNDNK.L
            **  *  **  ** **   ** * *** * ** * ***  * * ***** *    ** *
NL1-MOU   DSKSTVRELIEKIRSVFVDNLDELNWMDEESKKKAQEKAMNIREQIGYPDYILEDNNKHL
          **** ***** * * ***  **** ************** ****** ******  *  *
NL2-HUM   DSKSMVRELIDKVRTVFVETLDELGWMDEESKKKAQEKAMSIREQIGHPDYILEEMNRRL
            **  *  *              * ** *** * ** * *  * * **  *
NL3-HUM   ASKAKVQQLVEDIKYILGQRLEELDWMDAETRAAARAKLQYMMVMVGYPDFLLKPDA..V

                500        510        520        530        540        550
NEP-HUM   NNEYLELNYKEDEYFENIIQNLKFSQSKQLKKLREKVDKDEWISGAAVVNAFYSSGRNQI
            **  *   ** **** ****       ********* **  ********** ****
NL1-MOU   DEEYSSLTFYEDLYFENGLQNLKNNAQRSLKKLREKVDQNLWIIGAAVVNAFYSPNRNQI
          ***** * * ******* *****  ***** ******* ********************
NL2-HUM   DEEYSNLNFSEDLYFENSLQNLKVGAQRSLRKLREKVDPNLWIIGAAVVNAFYSPNRNQI
          * *        *  ** * *        * *  * *  **   *        ** * ** **
NL3-HUM   DKE.YEFEVHEKTYFKNILNSIRFSIQLSVKKIRQEVDKSTWLLPPQALNAYYLPNKNQM
```

Fig. 5 (cont'd)

```
                560        570        580        590        600        610
NEP-HUM   VFPAGILQPPFFSAQQSNSLNYGGIGMVIGHEITHGFDDNGRNFNKDGDLVDWWTQQSAS
          ************  *  *** ********************** * *  ***   **
NL1-MOU   VFPAGILQPPFFSKDQPQSLNFGGIGMVIGHEITHGFDDNGRNFDKNGNMLDWWSNFSAR
          ************** *** ******************************** *******
NL2-HUM   VFPAGILQPPFFSKEQPQALNFGGIGMVIGHEITHGFDDNGRNFDKNGNMMDWWSNFSTQ
          *********        ** ** **** **** *** ** *  *  **  **  *
NL3-HUM   VFPAGILQPTLYDPDFPQSLNYGGIGTIIGHELTHGYDDWGGQYDRSGNLLHWWTEASYS

                620        630        640        650        660        670
NEP-HUM   NFKEQSQCMVYQYGNFSWDLAGGQHLNGINTLGENIADNGGLGQAYRAYQNYIKKNGEEK
           *  ***** ******** **  *  **  ********** *** **        *
NL1-MOU   HFQQQSQCMIYQYGNFSWELADNQNVNGFSTLGENIADNGGVRQAYKAYLRWLADGGKDQ
          **  ** ******** ** *** ****** ********************** * * *****
NL2-HUM   HFREQSECMIYQYGNYSWDLADEQNVNGFNTLGENIADNGGVRQAYKAYLKWMAEGGKDQ
           *   **   * *          * ***  ******** **   ** ** **  * *
NL3-HUM   RFLRKAECIVRLYDNFT...VYNQRVNGKHTLGENIADMGGLKLAYHAYQKWVREHGPEH

                680        690        700        710        720        730
NEP-HUM   LLPGLDLNHKQLFFLNFAQVWCGTYRPEYAVNSIKTDVHSPGNFRIIGTLQNSAEFSEAF
           **** *   **** * ****** **** ** ********   *  * ***   *****
NL1-MOU   RLPGLNLTYAQLFFINYAQVWCGSYRPEFAVQSIKTDVHSPLKYRVLGSLQNLPGFSEAF
          * **** **  ********************* ********************** *  *
NL2-HUM   QLPGLDLTHEQLFFINYAQVWCGSYRPEFAIQSIKTDVHSPLKYRVLGSLQNLAAFADTF
          ** *  ** ***** **  **   *           ** * *  ******      *
NL3-HUM   PLPRLKYTHDQLFFIAFAQNWCIKRRSQSIYLQVLTDKHAPEHYRVLGSVSQFEEFGRVL

                740        750
NEP-HUM   HCRKNSYMNPEKKCRVW
          **    * * * * ** *
NL1-MOU   HCPRGSPMHPMKRCRIW
          ** ** **** *** * *
NL2-HUM   HCARGTPMHPKERCRVW
          **    ** *   * **
NL3-HUM   HCPKVSPMNPAHKCSVW
```

Fig. 6 (cont'd)

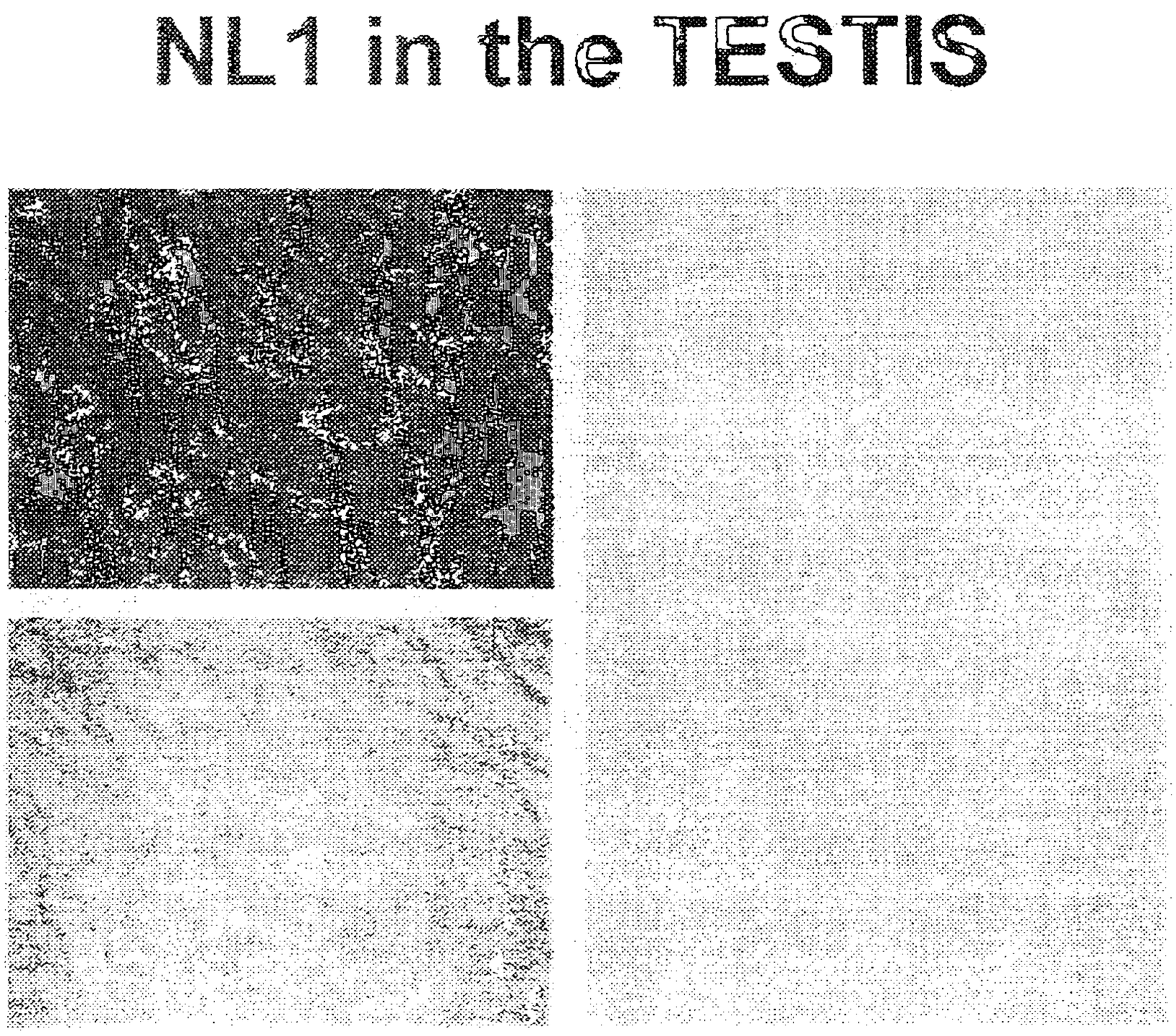
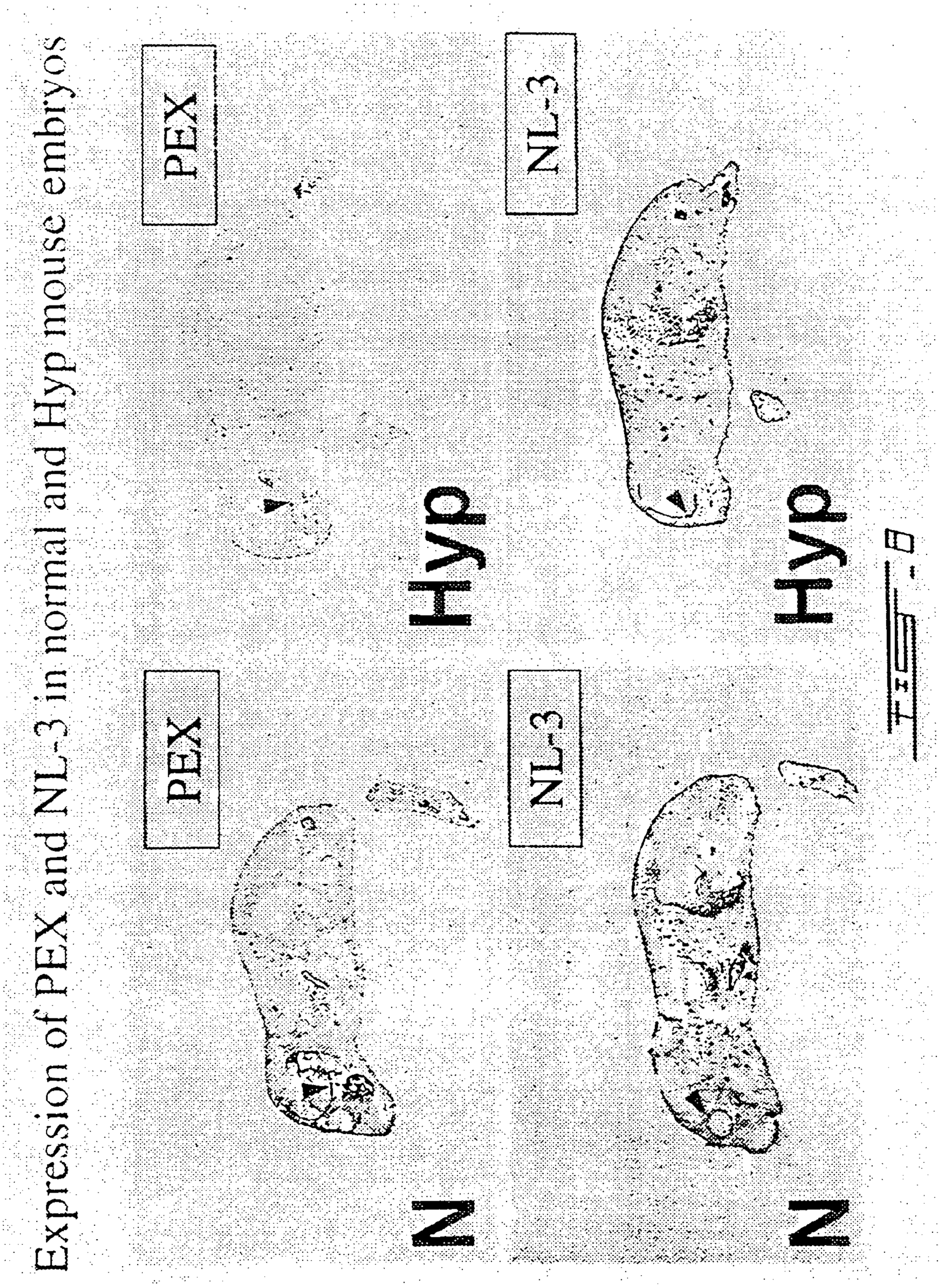


FIG. 7


Expression of PEX and NL-3 in normal and Hyp mouse embryos
PEX
NL-3
Hyp
Hyp
PEX
NL-3
N
N

NL3 in the BRAIN

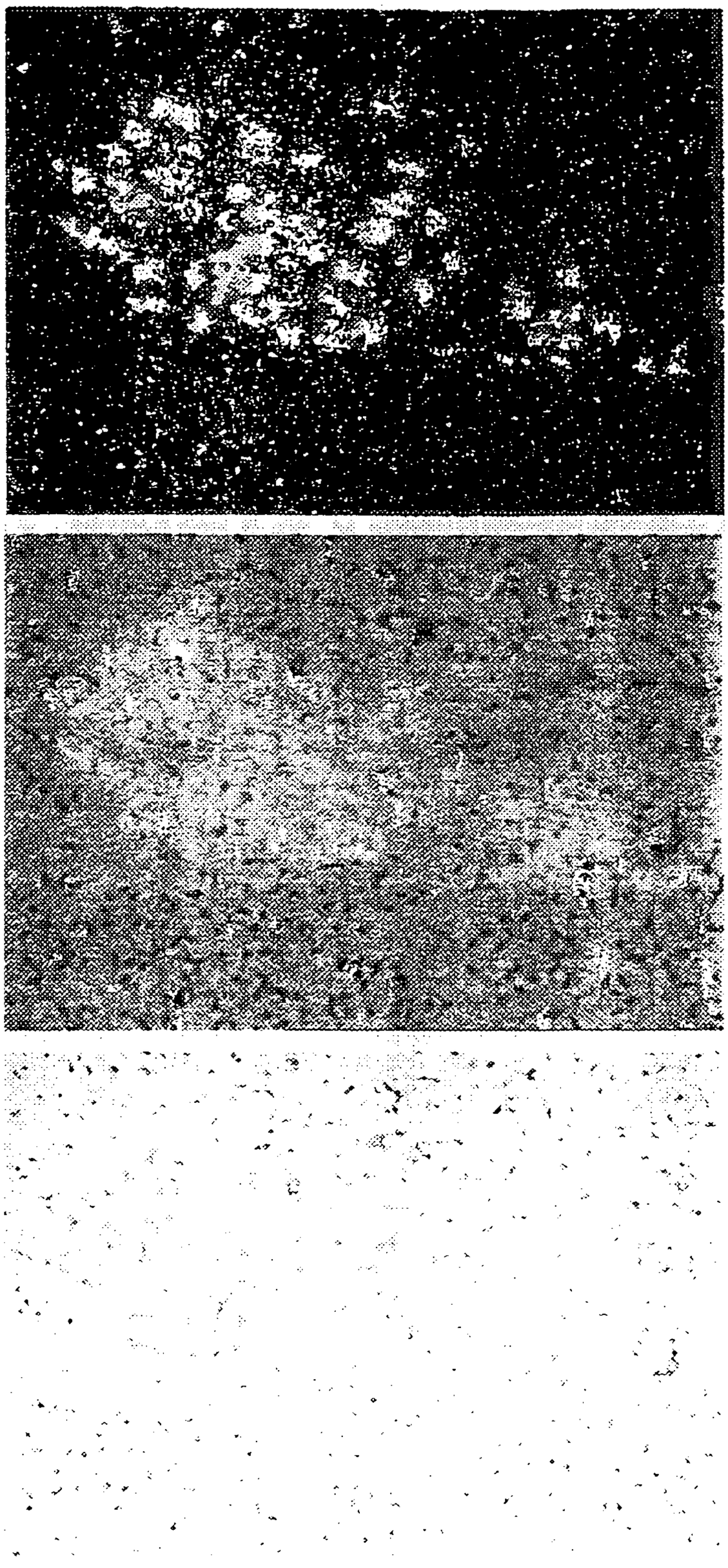


FIG. 9

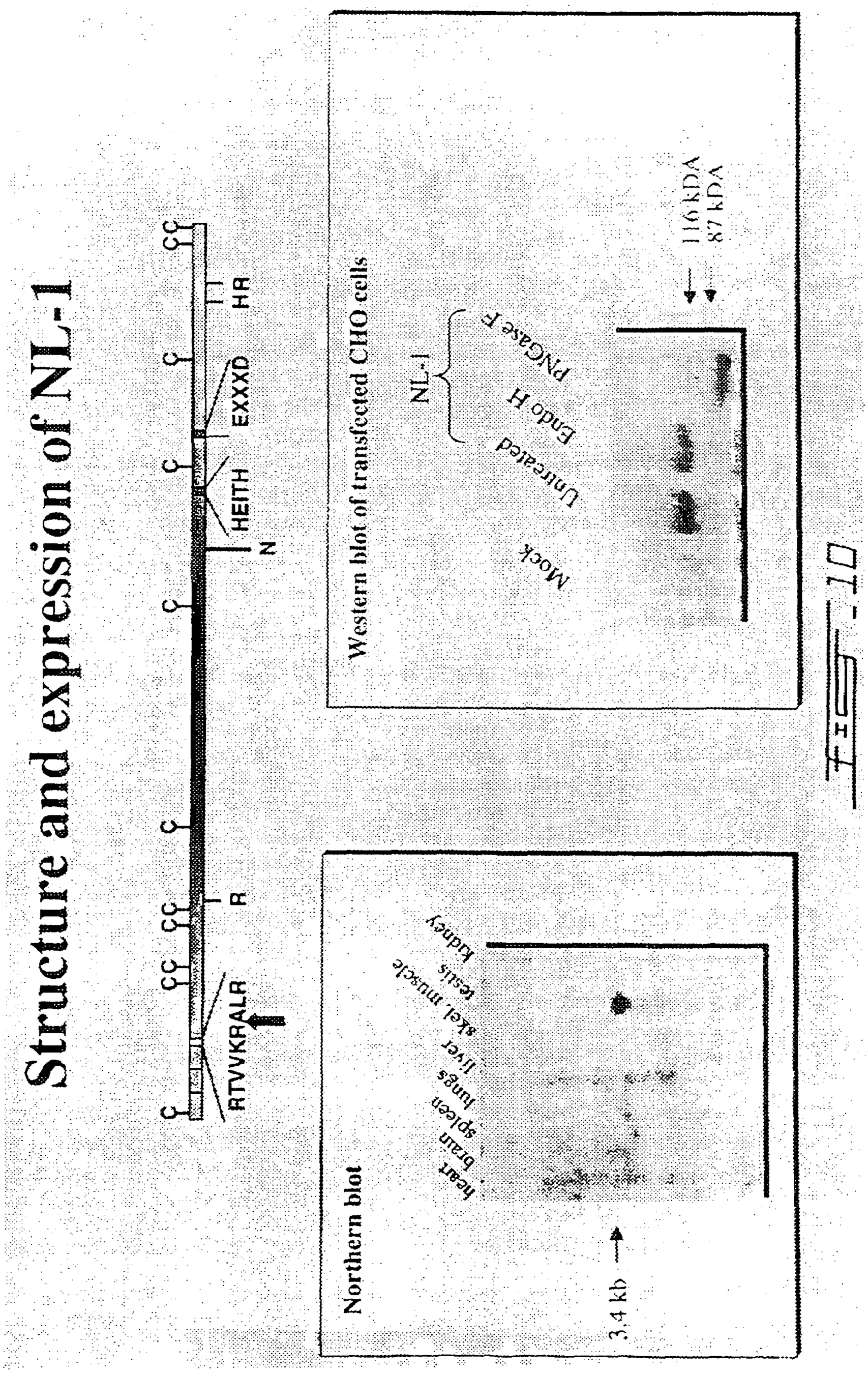

Structure and expression of NL-1
CC
C
C
C
CC
I
R
I
I
HR
EXXXD
HEITH
N
RTVVKRALR
Western blot of transfected CHO cells
Mock
Unrcated
Endo H
PNGase F
NL-1
116 kDA
87 kDA
Northern blot
heart
brain
spleen
lungs
liver
skel. muscle
kidney
3.4 kb

FIG. 10

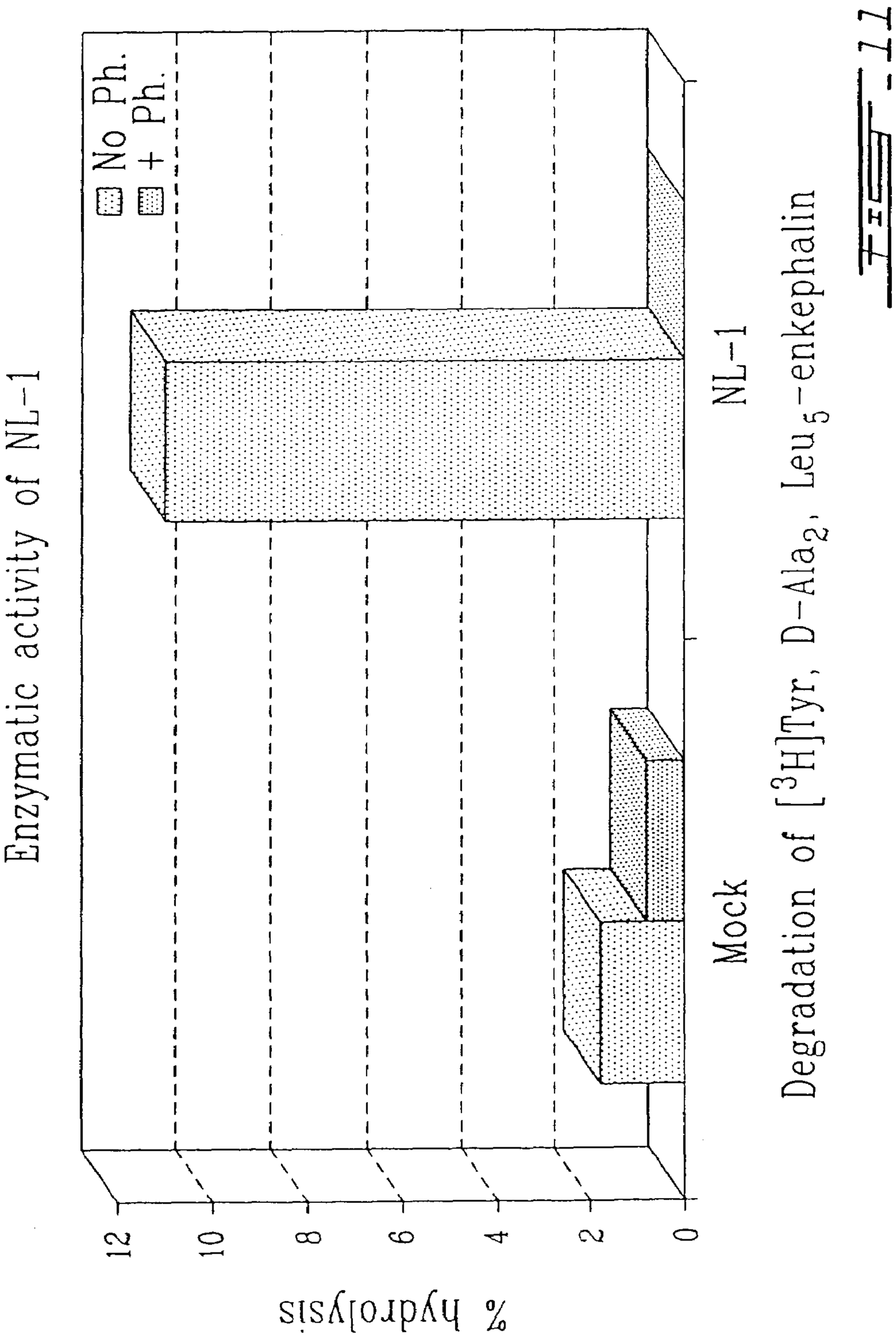

Degradation of [3H]Tyr, D-Ala2, Leu5-enkephalin
NL-1
Mock
No Ph.
+ Ph.
Enzymatic activity of NL-1
% hydrolysis
0
2
4
6
8
10
12

FIG - 11

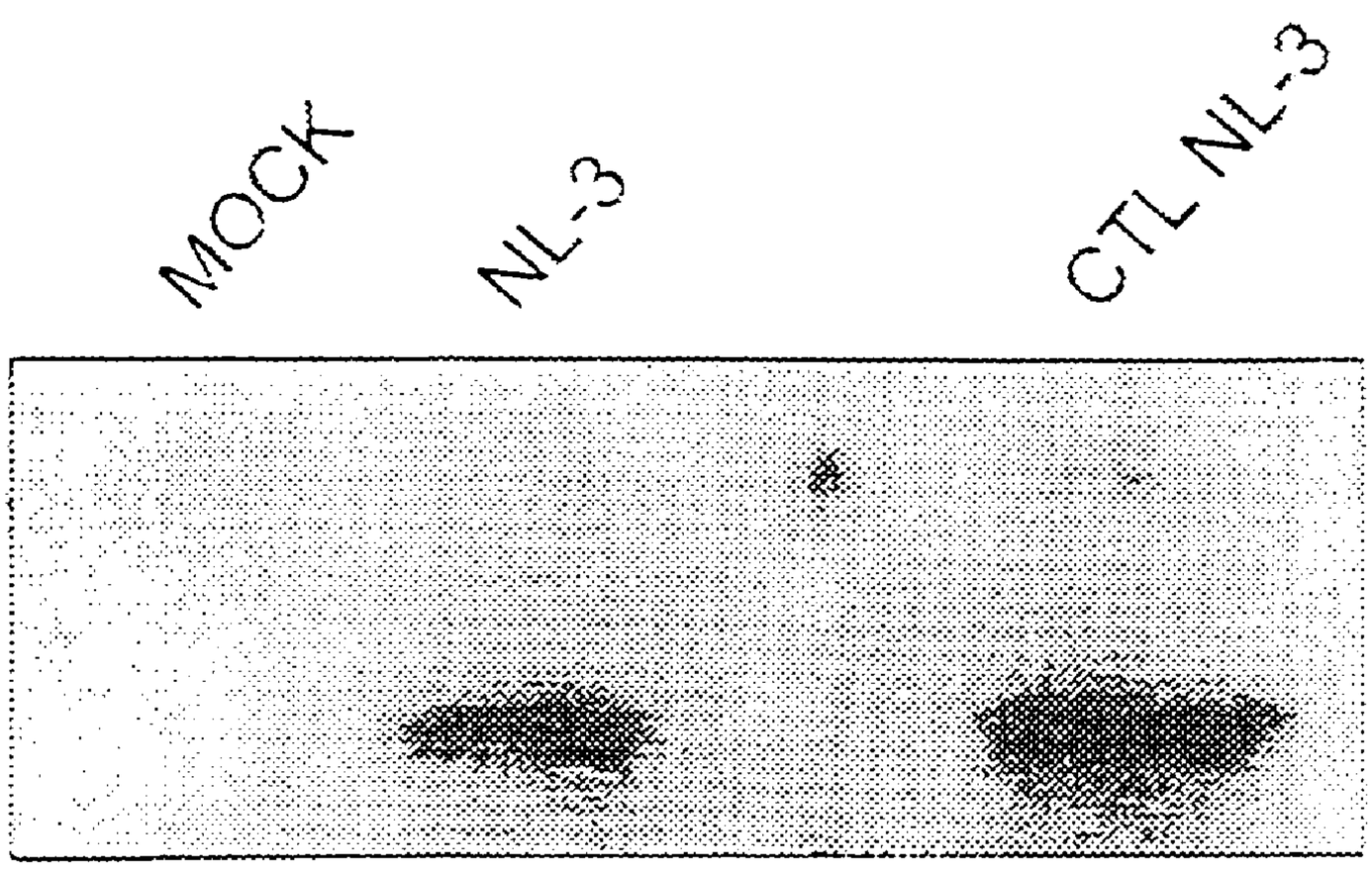


FIG. 12

METALLOPROTEASES OF THE NEPRILYSIN FAMILY

This application is the U.S. National Phase of International Application PCT/CA00/00147, filed Feb. 11, 2000, published in English under PCT Article 21(2), which designated the U.S. PCT/CA00/00147 claims priority to Canadian Patent Application No. 2,260,376, filed Feb. 11, 1999. The entire content of these applications are incorporated herein by reference.

BACKGROUND OF THE INVENTION

Peptides are used by cells from yeast to mammals to elicit physiological responses. The use of peptides as messengers usually involves the following steps: 1) production and release of the peptide by a specific cell, 2) interaction of the peptide with a receptor on the surface of the target cell, and 3) degradation of the peptide to terminate its action. The first and last steps of this scheme require the participation of proteases/peptidases. There is increasing evidence that membrane-associated zinc-metallopeptidases play important roles in both of these steps. Although activation of prohormone precursors into bioactive peptides is generally performed by proteases of the subtilisin family located in the Trans-Golgi Network or in secretory granules of the cell (for a review see: (Seidah and Chretien, 1995)) a few peptides need a final processing step. This step involves the action of membrane-associated zinc-metallopeptidases. Two cases are particularly well documented: angiotensin-converting enzyme (ACE) which cleaves inactive angiotensin I into angiotensin II (Corvol and Williams, 1997) and endothelin-converting enzymes (ECEs) which cleave isoforms of big endothelins into endothelins (Turner, 1997a). In addition to their role in peptide activation, cell surface zinc-metallopeptidases have also been implicated in the termination of the peptidergic signal by breaking down the active peptides into inactive fragments. One of the best known of these peptidases is probably Neutral Endopeptidase-24.11 (Neprilysin, NEP) that has been implicated in the physiological degradation of several bioactive peptides (Kenny, 1993). Interestingly, NEP and the ECEs show significant structural similarities and appear to be members of a family of peptidases that also includes PEX, a newly discovered and not yet characterized peptidase, and the KELL blood group protein (Turner and Tanzawa, 1997b). Because of their important role as regulators of bioactive peptide activity, these enzymes (more specifically NEP and the ECEs) have been identified as putative targets for therapeutic intervention, similar to the way ACE inhibitors are used to control blood pressure. The recent discovery of PEX, another member of the family, which appears to be involved in phosphate homeostasis, raised the possibility that other yet unknown members might exist.

Members of the NEP-like family are type II membrane proteins consisting of three distinct domains: a short NH2-terminal cytosolic sequence, a single transmembrane region, and a large extracellular or ectodomain responsible for the catalytic activity of the enzyme. There are potential N-glycosylation sites and cysteine residues that are involved in disulfide bridges stabilizing the conformation of the active enzyme. These enzymes are metalloenzymes with a Zn atom in their active site. As such, they belong to the zincin family of peptidases which is characterized by the active site consensus sequence HEXXH (Hooper, 1994), where the two histidine residues are zinc ligands. In members of the NEP-like family of peptidases, the third zinc ligand is a

glutamic acid residue located on the carboxy-terminus side of the consensus sequence. This characteristic puts them in the gluzincin sub-family (Hooper, 1994). The model enzyme for gluzincins is thermolysin (TLN) a bacterial protease whose 3D structure has been determined by X-ray crystallography (Holmes and Matthews, 1982). The active site of NEP has been extensively studied by site-directed mutagenesis and several residues involved in zinc binding (Devault et al., 1988b; Le Moual et al., 1991; Le Moual et al., 1994), catalysis (Devault et al., 1988a; Dion et al., 1993), or substrate binding (Vijayaraghavan et al., 1990; Beaumont et al., 1991; Dion et al., 1995; Marie-Claire et al., 1997) have been identified (for a recent review see Crine et al., 1997).

SUMMARY OF THE INVENTION

Here, we developed an RT-PCR strategy to look for other members of this important family of peptidases. This strategy allowed the molecular cloning and characterization of three additional NEP-like (NL) metallopeptidases (called NL-1, NL-2 and NL-3). Knowledge obtained through these studies allows the generation of reagents (nucleic acid probes and primers, antibodies and active recombinant enzymes) for further biochemical characterization of these enzymes and their pattern of expression and will greatly help the rational design of specific inhibitors that could be used as therapeutic agents.

Accordingly, the present invention relates to the following products:

A. Degenerate primers for screening new NEP-related enzymes;
B. NL-1, NL-2 and NL-3 proteins as NEP-related enzymes;
C. Nucleic acids encoding these enzymes;
D. Antibodies directed against the enzymes;
E. Recombinant vectors comprising the nucleic acids encoding the enzymes and hosts transformed therewith;
F. Fragments of the nucleic acids useful as probes or primers to hybridize and detect the presence of an NL-1, NL-2 and NL-3 genes, or to hybridize and amplify and produce gene fragments;
G. Soluble forms of NL-1, NL-2 and NL-3; and
H. Nucleic acids comprising the N-terminal part of NL-1 or NL-2 which terminates with a sequence encoding a furin recognition site, such nucleic acids being useful for making a fusion protein with the ectodomain of any protein of interest, and for releasing a soluble form of that protein of interest (containing the ectodomain) in the medium.

Also the present invention relates to the following methods:

A. A method for screening NEP-related enzymes that make use of degenerate primers or probes selected from a region of NEP family members in a highly conserved region, namely around the zinc-binding sites; and
B. A method for producing NL-1, NL-2 or NL-3 that includes the steps of culturing the above recombinant host and recovering NL-1, NL-2 and NL-3 gene products therefrom.

The present invention will be described hereinbelow by referring to specific embodiments and appended figures, which purpose is to illustrate the invention rather than to limit its scope.

In the first section, general procedures leading to the identification and localization of NL-1, NL-2 and NL-3 are given. In the second section, slightly different procedures are given for completing or reiterating the work performed on NL-1.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1**: Amino acid sequence comparison of human NEP (SEQ ID NO: 1), PEX (SEQ ID NO: 2), KELL (SEQ ID NO: 3) and ECE1 (SEQ ID NO: 4) peptidases. Amino acid sequences in boxes are those used to design the oligonucleotide primers. Numbers and arrows under the sequences identify the primer and its orientation.

FIG. **2**: Sequences of the oligonucleotide primers used in the PCR reactions (1A: SEQ ID NO: 5; 1B: SEQ ID NO: 6; 2A: SEQ ID NO: 7; 2B: SEQ ID NO: 8; 3: SEQ ID NO: 9; 4: SEQ ID NO: 10; and 5: SEQ ID NO: 11).

FIG. **3**: Nucleotide (SEQ ID NO: 12) and amino acid (SEQ ID NO: 13) sequence of the mouse NL-1 cDNA. The sequence of the DNA fragment obtained by PCR is in brackets.

FIG. **4**: Partial nucleotide (SEQ ID NO: 14) and amino acid sequence (SEQ ID NO: 15) of the human NL-2 cDNA. The sequence of the DNA fragment obtained by PCR is in brackets.

FIG. **5**: Partial nucleotide (SEQ ID NO: 16) and amino acid sequence (SEQ ID NO: 17) of the human NL-3 cDNA.

FIG. **6**: Amino acid sequence comparison of NEP (SEQ ID NO: 1), NL-1 (SEQ ID NO: 13), NL-2 (SEQ ID NO: 15) and NL-3 (SEQ ID NO: 17) peptidases.

FIG. **7**: In situ hybridization of mouse testis sections using NL-1 as a probe.

FIG. **8**: In situ hybridization of mouse sections using mouse NL-3 as a probe.

FIG. **9**: In situ hybridization of mouse spinal chord sections

FIG. **10**: Expression of NL-1 in mammalian cells.

FIG. **11**: Activity of recombinant soluble NL-1.

FIG. **12**: Expression of a soluble form of NL-3 using NL-1 amino-terminal domain.

DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE INVENTION

Section 1)

Materials and Methods

DNA and RNA manipulations

All DNA manipulations and Northern blot analysis were performed according to standard protocols (Ausubel et al., 1988; Sambrook et al., 1989).

mRNA purification and cDNA synthesis

mRNAs were prepared from mouse testis using Quick Prep Micro mRNA purification kit (Pharmacia Biotech). Purified mRNAs were kept at −70° until ready used. First strand cDNA was synthesized from 1 μg of mRNA using the First-Strand cDNA synthesis kit (Pharmacia Biotech). The human testis cDNA library was obtained from Clonetech.

Polymerase chain reaction protocol

PCR was performed in a DNA thermal cycler with 5 μl of cDNA template and 1 μl of Taq DNA polymerase in a final volume of 100 μl, containing 1 mM $MgCl_2$, 2 μM of each primer oligonucleotide, 20 μM of each dNTP and 5% DMSO. Cycling profiles included an initial denaturation step of 5 min at 94° C., followed by 30 cycles of 1 min at 94° C., 1 min at 40° C. and 1.5 min at 72° C. A final extension step was performed at 72° C. for 10 min. The amplified DNA was loaded on a 2% agarose gel and visualized by staining with ethidium bromide. Fragments ranging in size between 500–700 bp were cut and eluted from the gel. If needed, a second round of PCR was done with nested oligonucleotide primers, using 10 μl of the first PCR reaction, or of the eluted band cut from the agarose gel. Resulting fragments were ligated in pCR2.1 vector (Invitrogen) according to the distributor's recommendations. DH5α *Escherichia coli* cells were transformed with the ligation mixture and grown on 2YT plates in the presence of kanamycin. Plasmids were prepared from resistant cells and sequenced.

In situ hybridization on mouse tissues and chromosomal localization of human genes

In situ hybridization on whole mouse slices or isolated tissues was performed as described previously (Ruchon et al., 1998).

To determine the chromosomal localization of human NL-2 and NL-3 genes, a technique for mapping genes directly to banded human chromosomes was used. Metaphase chromosomes were obtained from lymphocytes cultured from normal human peripheral blood. Cells were synchronized with thymidine and treated with 5-bromodeoxyuridine (BrdU) during the last part of the S phase to produce R-banding. Biotin-labeling of the probe was done by nick-translation (Bionick, BRL) and the probe was visualized by indirect immunofluorescence.

Antibody production

To raise antibodies against the new peptidases, the cDNA sequences of each protein was compared to that of other members of the family and the sequence segment showing the less homology was used. These sequences are from amino acid residues 273 to 354 for NL-1, from 75 to 209 for NL-2 and from 143 to 465 for NL-3. These cDNA fragments were cloned in vector pGEX2T (Pharmacia Biotechnology) downstream from and in phase with Gluthatione-S-transferase (GST). Plasmids were transformed in *E. coli* strain AP401 and, induction of synthesis and purification of the fusion proteins were performed as recommended by the supplier. The NL polypeptides were cleaved from the fusion protein with thrombin and purified by SDS-PAGE. NL polypeptides were injected to rabbits or mice according to the following schedules: for rabbits, initial injection of 150 μg of protein with boosts of the same amount 4 weeks and 8 weeks following the initial injection; for mice, initial injection of 100 μg of protein followed by boosts of the same amounts 3 and 6 weeks later. A month after the last injection, sera were collected from the animals and tested by immunoblotting against the initial *E. coli*-produced antigens and the recombinant proteins produced in mammalian cell lines.

Production of monoclonal antibodies

cDNA fragments corresponding to amino acids segments of NLs selected to raise antibodies were used to construct a GST-fusion protein in *E. coli*. This fusion protein was purified from *E. coli* extracts by affinity chromatography on a glutathione-Sepharose column according to the supplier's instructions (Amersham-Pharmacia). After thrombin cleavage, the NL portion of the GST fusion protein was further purified by electroelution from a polyacrylamide gel. This material was used to immunise 4 mice (5 injections of ≈50 μg of NL polypeptide). Blood was collected from each mice after the immunisation schedule and the presence of antibodies in mice serum was assessed by ELISA using microtiter plates coated with NL polypeptide from *E. coli* extracts. Mice sera were also tested for the presence of NL antibodies by Western blotting extracts of mammalian cells transfected with the NL expression vectors. One mouse selected for its high titer of NL specific antibodies (as measured by ELISA)

was sacrificed and its spleen cells were collected and immortalised by fusion with myeloma cells(strain: P3-X63Ag.653 from ATCC) as described previously (Crine 1985). Hybridoma cells were selected for their ability to grow in HAT selection medium and cloned by several rounds of limiting dilution. Hybridomas showing proper affinity and specificity to the enzymes NL-1, NL-2 and NL-3 where selected.

Expression of NLs in cultured mammalian cells and enzymatic assays The cDNAs for NL-1 and NL-3 were cloned in vectors pcDNA3 or pRcCMV (Invitrogen) and introduced by transfection in mammalian cell lines according to procedures already described in our laboratory (Devault et al., 1988a). Procedures to prepare extracts of cellular proteins or culture media were also described in previous papers (Devault et al., 1988a; Lemay et al., 1989). The presence of NLs in these extracts was monitored by immunoblotting using specific antibodies.

Extracts of cellular proteins and culture media were assayed for enzymatic activity. Two tests were performed. The first used [^{3}H]-Tyr-(D)Ala$_2$-Leu-enkephalin as substrate and was performed according to Lemay et al., (1989). The second used bradykinin as substrate and was performed as described by Raut et al. (1999).

Results

Cloning of NL-1, a new member of the NEP family

The molecular cloning in the past few years of ECEs, PEX and KELL showed that all these proteins have between 50 and 60% similarity with NEP. This observation led us to believe that these peptidases are part of an extended family and that there could be still additional members to be discovered. To test this hypothesis, we aligned the amino acid sequences of the members of the NEP-like family and designed degenerate oligonucleotide primers to be used in RT-PCR reactions (FIGS. **1** and **2**). These primers were located on either side of the HEXXH consensus sequence for zincins. Because they are highly degenerate, primers 1 and 2 were each subdivided into two pools, 1A-1B, and 2A-2B, respectively (FIG. **2**). Any PCR amplified DNA fragment that corresponds to a peptidase of the family should normally contain the consensus sequence and be easily recognized by sequencing of the cloned fragments. Using this strategy, we first performed PCR reactions with primer pairs **1**A-**3** and **1** B-**3**. The amplified DNA migrates mostly as a smear starting at around 700 bp and going down to 100 bp. As the expected fragments should be around 550 bp, we isolated from the gel the section corresponding to DNA fragments longer than 500 bp. A second round of PCR reactions was performed with both crude PCR products of the first reaction and isolated DNA bands, using primers 2A-3 and 2B-3. The expected 296 bp fragment was seen on the gel (not shown).

Cloning of these DNA fragments generated approximately 350 clones, of which 44 were sequenced. Nine of these had no inserts or corresponded to sequences not related to the NEP family, 24 corresponded to NEP, 3 to PEX, and 8 corresponded to one putative new member of the family, since they all contained the HEXXH consensus sequence for zincins and showed 65% homology with mouse NEP (in boxes FIG. **3**). This fragment was then used to screen a mouse testis cDNA library, and allowed us to isolate a complete cDNA of 2592 nucleotides (FIG. **3**). The identity of this sequence with other members of the family is presented in Table I. This new member was called NL-1, for NEP-like peptidase 1.

Cloning of NL-2 and NL-3

A strategy similar to that described for amplification of enzymes of the NEP family from mouse testis cDNAs was used with a human testis cDNA library using two different oligonucleotide primers. This time, DNA fragments of approximately 900 bp were obtained and cloned. Ten clones were sequenced, revealing the presence of NEP and two new peptidases of the family that we have called NL-2 and NL-3.

The NL-2 PCR fragment was 879 nucleotides in length and encoded a 293 amino acid residue segment probably located in the carboxy-terminal domain of this putative peptidase (in brackets FIG. **4**). This PCR fragment was then used to screen a lambda gt10 human brain cDNA library. It allowed the isolation of other cDNA fragments which overlap partially with the NL-2 PCR fragment. Fusion of these lambda clones and the PCR fragment resulted in an open reading frame of 770 amino acid residues. The use of 5' RACE protocols with human testis cDNA libraries allowed completion of the sequence of NL-2 ORF (FIG. **4**). This ORF codes for a putative protein that is about 80% identical to the mouse NL-1 protein (FIG. **6**). Across species, members of the NEP, PEX, ECEs sub-families have highly conserved sequences (more than 94% identity). Although a sequence identity of about 80% only exists between the novel human protein and mouse NL-1, these proteins share unique characteristics that make possible the fact that NL-2 protein may be the human homologue of NL-1. The identity of NL-2 with other members of the family is presented in Table I.

The 879 bp PCR fragment encoding NL-3 showed an open reading frame of 293 amino acid residues (FIG. **5**, in brackets). Sequence analysis of NL-3 showed that it was 94.2% identical to an EST sequence from mouse embryonic tissue present in publicly accessible DNA data banks. This mouse EST sequence, commercially available from American Tissue and Cell Culture (ATCC), had been obtained previously by our laboratories.

Since Northern blot analysis of human tissues with the NL-3 PCR fragment showed the expression of this protein in spinal chord (see below), the same PCR DNA fragment was used to screen by hybridization a human spinal chord cDNA library constructed in phage A vectors. One clone contained a full-length ORF of 752 amino acid residues that encompassed the 293 amino acid residue ORF of the PCR fragment. Further probing, cloning and sequencing lead to the obtention of NL-3 full sequence, shown in FIG. **5**.

FIG. **6** presents a comparison of the amino acid sequence of the new NEP-like enzymes and Table I shows the extent of identity between members of the family.

Cellular distribution of NL-1, NL-2 and NL-3 peptidases

Determining the tissue distribution of NL-1, NL-2 and NL-3 may provide clues to identify the peptidergic systems in which they are involved. It will be particularly interesting to compare the tissue distribution of these peptidases with that of NEP and the ECEs to determine whether or not the physiological functions of NL-1 and/or NL-2 and/or NL-3 may overlap those of NEP and/or ECEs.

In situ hybridization (ISH), using our mouse cDNA, was used to determine the spatial and temporal expression of NL-1 during mouse development, as done previously for PEX (Ruchon et al., 1998)). Serial sections of whole foetal (12, 15 and 19 dpc) and adult mice (1, 3 and 6 days old) were hybridized with an [^{35}S]-labeled RNA probe. FIG. **7** shows a section of mouse testis which was the only tissue identified to express NL-1 by this technique. Cells of seminiferous tubules are specifically labeled but spermatids located near

the center of the tubule showed strongest labeling. These cells are in the last stage of maturation into spermatozoids. The presence of NL-1 in testis has now been confirmed by Northern analysis of mouse tissues (see FIG. **10**). Other tissues express NL-1, when analyzed by RT-PCT, which is a more sensitive assay (not shown).

A similar approach was used to determine the localization of NL-3 using the mouse EST obtained from ATCC. FIG. **8** shows sections of whole mouse at 17 days of embryonic development and 4 days post-natal. Several tissues are expressing this putative peptidase including brain, where it is associated with neurons (FIG. **9**), spinal chord, liver, spleen and bones. Labeling was stronger in bones from Hyp mouse, an animal model for hypophosphatemic rickets (FIG. **8**). In bones, NL-3 was found to be expressed by osteoblasts (not shown).

Northern blotting experiments were performed on several tissues with NL-2 and NL-3 probes. A Human Multiple Tissues Northern Blot (Clontech) was hybridized with specific probes. A single RNA band of approximately 4.0 kb was revealed by the probe for NL-2. Expression of NL-2 is restricted to brain and spinal cord (not shown). However, RT-PCR has shown the presence of this enzyme in testis (not shown).

A single RNA band of approximately 3.0 kb was detected with the specific probe for NL-3 (not shown). NL-3 expression was observed mainly in ovary, spinal cord and adrenal gland.

Chromosomal localisation of the human gene for NL-2 and NL-3

As a mean to get clues on the function of the new metallopeptidases in vertebrates, we have localized the new cDNAs on human chromosomes, in order to look for a possible link between the gene locus and mapped genetic diseases in humans. To do so, we have mapped the NL-2 and NL-3 genes by high-resolution fluorescence in situ hybridization (FISH). NL-2 was localized to chromosome band 1p36. Consistent with the cellular distribution of NL-2 in humans, genetic diseases of the CNS such as dyslexia, neural tube defect, neuroblastoma, neuronal type of Charcot-Marie-Tooth disease have all been mapped in this region and represent potential targets for a role of NL-2 in humans. NL-3 was localized to chromosome band 2q37. Consistent with a role of NL-3 in bones, a form of Albright hereditary osteodystrophy was mapped to the same chromosomal locus (Phelan et al., 1995).

In view of the foregoing, NL-2 and NL-3 are metallopeptidases that are assumed to be immediately useful as markers for a disease or disorder associated with human chromosomal locus 1p36 and 2q37, respectively. Their localization on a chromosome band associated with known diseases suggests that they may be expressed or co-expressed with one or more genes, as a cause or a consequence of disease development. It is possible that these enzymes are up or down regulated, alone or along with other genes involved in a disease. Therefore, antibodies or other ligands specific to NL-2 or NL-3 may be used for a diagnostic purpose, as well as primers or probes in diagnostic assays using nucleic acid hybridization or amplification techniques. Otherwise, primers or probes directed against the nucleic acids of NL-2 and NL-3 would be useful to map the mutations of a gene located in close proximity and involved in the disease. Therefore, no matter which exact function NL-2 and NL-3 gene products have, their chromosomic localization provides one diagnostic utility. This localization as well as the tissular distribution provide information as to the disease and tissue to be investigated to elucidate the exact function of these enzymes.

NL-1 resembles NL-2, sharing with the latter about 80% homology in the amino sequence and sharing structural characteristics such as the furin recognition sequence located at the proximal end of the ectodomain. NL-2 might be the human homologue of mouse NL-1. If such was the case, these two proteins would have a substantial degree of divergence and, maybe, different profiles of activity varying from one species to another.

Chromosomal localization of NL-1 was determined in mouse genome by Single Strand Conformational Polymorphism (SSCP) in collaboration with The Jackson Laboratory Backcross DNA Panel Mapping Resource. NL-1 was localized to the distal region of mouse chromosome 4 which corresponds to human chromosome region 1p36 where is located NL-2 gene. This reinforces our hypothesis that NL-1 and NL-2 are species variants.

Production of antibodies against NLs

Antisera collected from injected animals were first tested by immunoblotting on GST-antigen fusion proteins produced in *E. coli.* Antiserum from one rabbit recognized the NL-1 -related polypeptide and antisera from one mouse and one rabbit reacted with the NL-3-related polypeptide (results not shown). The anti NL-1 antiserum and the mouse anti NL-3 antiserum, which appeared more specific than the rabbit antiserum, were next tested by immunoblotting on extracts of proteins and culture media from cells expressing NL-1 or NL-3 (see below).

Expression of NL-1 in CHO cells

The cDNA encoding the full-length NL-1 protein was cloned in the mammalian expression vector pcDNA**3**-RSV and transfected in CHO cells. Stable cell lines were established by selection with the drug G418 and tested by immunoblotting for the presence of NL-1.

Small amounts of NL-1 were found in the extracts of transfected CHO cells (results not shown). This intracellular species was sensitive to endo H digestion, indicating that the sugar moiety was not mature and suggesting ER localization (results not shown). The culture medium of transfected CHO cells showed the presence of soluble NL-1 (FIG. **10**). This extracellular species was resistant to endo H suggesting true transport through the late secretory pathway. The cDNA sequence of NL-1 predicts a type II transmembrane protein. The mechanism by which NL-1 is transformed into a soluble protein is not known presently. However, examination of the amino acid sequence revealed the presence of a putative furin cleavage site from residue 58 to 65 (FIG. **3**). A similar site is present in NL-2 sequence.

The soluble form of NL-1 was assayed for activity using [^{3}H]-Tyr-(D)Ala$_2$-Leu-enkephalin and bradykinin as substrates. FIG. **11** shows that NL-1 can degrade the enkephalin substrate (K_m=18±10 μM) and that this activity can be inhibited by phosphoramidon (IC_{50}=0.9±0.3 nM) and thiorphan (K_m=47±12 nM), a general inhibitor of enzymes of the NEP family. Bradykinin is also a substrate for NL-1 (not shown).

Use of NL-1 amino-terminal domain to promote secretion

The observation that NL-1 ectodomain was secreted, possibly through cleavage of the transmembrane segment by furin, raised the possibility to promote secretion of exogenous proteins that could be spliced to NL-1 amino-terminal domain (from initiator methionine to the furin site). To test this hypothesis, the ectodomain of NL-3 (from the third

cysteine to the end) was spliced to NL-1 amino-terminal domain using a PCR strategy and the recombinant DNA cloned in expression vector pRcCMV. The fusion protein was expressed by transfection of the vector in COS-1 and HEK 293 cells. The culture media of transfected cells was analyzed by immunoblotting using the mouse antiserum against NL-3. FIG. **12** shows the presence of NL-3 in the spent culture media of both COS-1 and HEK 293 cells. This result shows that NL-1 amino-terminal domain can be used to promote secretion of exogenous proteins.

The soluble form of NL-3 was assayed for activity using [^{3}H]-Tyr-(D)Ala$_2$-Leu-enkephalin as substrate. No activity was found.

The previous experiment showed that it was possible to use the amino-terminal domain of NL-1 to promote secretion of an otherwise membrane attached protein ectodomain. To verify whether the same strategy could be used to promote secretion of small peptides, a PCR strategy was used to splice human β-endorphin to the amino-terminal domain of NL-1 and the recombinant DNA was cloned in vector pRcCMV. The fusion protein was expressed by transfection of the vector in COS-1 and HEK 293 cells. The culture media of transfected cells was collected 48 h after transfection and the peptides purified as described previously (Noël et al., 1989). The presence of β-endorphin in the extracts was detected by radioimmunoassay. The results showed that both COS-1 and HEK 293 cells produced approximately 100 pg of β-endorphin per ml of culture medium. Therefore, the N-terminus of LN-1 and NL-2 which ends with a furin-recognition site will be useful to produce the soluble form of a protein of interest.

Section 2)

Materials and Methods

DNA manipulations

All DNA manipulations, phage library screening, and plasmid preparations were performed according to standard protocols (Ausubel 1988; Sambrook 1989). Site-directed mutagenesis was performed using a PCR-based strategy as described previously (Le Moual 1994).

mRNA purification and RT-PCR protocol for identification of new members of the neprilysin family

mRNAs were prepared from mouse testis using Quick Prep Micro mRNA purification kit (Pharmacia Biotech). First strand cDNA was synthesized from 1 μg of mRNA using the First-Strand cDNA synthesis kit (Pharmacia Biotech).

Two sense primers, oligonucleotide 3817 (5'-TGGATG-GAT/CGA/CIGG/AIACIA/CA-3') and oligonucleotide 3719 (5'-A/GTIGTITTT/CCCIGCIGGIA/GT/AIC/TTG/CCA-3') corresponding respectively to amino acid residues 459 to 465 and 552 to 560 of NEP sequence, and one antisense primer, oligonucleotide 3720 (5'-AIICCICCIA/TC/TA/GTCIGCIG/AC/TA/GTTT/CTC-3') corresponding to amino acid residues 646 to 654 (see FIGS. **1** and **2**), were synthesized. PCR was performed with 5 μl of cDNA template and 1 μl of Taq DNA polymerase in a final volume of 100 μl, containing 1 mM $MgCl_2$, 2 μM of each oligonucleotide 3817 and 3720, 200 μM of each dNTP and 5% DMSO. Cycling profiles included an initial denaturation step of 5 min at 94° C., 30 cycles of 1 min at 94° C., 1 min at 40° C. and 1.5 min at 72° C., and a final extension step at 72° C. for 10 min. One half of the amplified DNA was fractionated on a 2% agarose gel and fragments ranging in size between 500–700 bp were purified and resuspended in a final volume of 50 μl. A second round of PCR was done with primers 3719 and 3720, using as template either 10 μl of the first PCR reaction or 5 μl of the purified fragments, and the new PCR products were ligated in pCR2.1 vector (Invitrogen). Several identical clones corresponded to a potential new member of the NEP family. We called this member NL1 for NEP-like 1.

Cloning of full-length NL1 cDNA

The cloned NL1 PCR fragment was used as probe to screen a mouse testis λ Uni-ZAP™XR cDNA library (Stratagene). Twelve out of a hundred positive phages were plaque purified and subcloned into pBS SK vector (Stratagene). As the longest clone analyzed presented an incomplete ORF (pBS-NL1A), 5'RACE with primers located in vector (5'-TAGTGGATCCCCCGGGCTGCAG-3', sense primer) and NL1 (5'-ACCAAACCTTTCCTGTAGCTCC-3', antisense primer, nt 1303 to 1324 of NL1; was subsequently performed on the DNA of the remaining semi-purified positive clones. Amplification was performed with 1 μl of Vent polymerase in a final volume of 100 μl containing 50 ng of DNA, 4 mM of $MgSO_4$, 1 μM of each oligonucleotide, 200 μM of each dNTP and 10% DMSO. Cycling parameters included an initial denaturation step of 1 min at 94° C., 25 cycles of 30 sec at 94° C., 30 sec at 60° C. and 1 min at 72° C., and an incubation of 10 min at 72° C. A PCR fragment of the expected length was subcloned into pCR2.1 vector (clone pCR-NL1A), but sequencing revealed no initiator ATG codon. A nested 5'RACE was then performed on mouse testis cDNA using the Marathon Ready cDNA kit (Clontech) with sense oligonucleotides AP1 and AP2 (from the kit) and NL1 antisense oligonucleotides 5'-CCTGAGGGCTCGTTTTACAACCGTCCT-3' (nt 503 to 529 of NL1) and 5'-CTCATCCCAGGAGAAGTGTAG-CAGGCT-3' (nt 475 to 502 of NL1) as recommended by the supplier. The resulting fragment was cloned into pCR2.1 vector (pCR-NL1B). Since only ten bp were missing for the initiator ATG codon, we reconstructed the 5' end of the cDNA by PCR-amplifying clone pCR-NL1A with sense primer 5'-CCACC ATGGTGGAGAGAGCAGGCTGGTGTCGGAAGAAG-3' (nt 332 to 364 of NL1; the 10 missing nucleotides are underlined) and antisense primer 5'-ACCAAACCTTTCCT-GTAGCTCC-3' (nt 1303 to 1324 of NL1) using Vent polymerase as described above. The DNA fragment was then inserted into pCR2.1 (clone pCR-NL1C). The entire ORF was reconstituted following digestion of pBS-NL1A and pCR-NL1C with EcoRI and PflMI. The 5' end of NL1 cDNA was excised from pCR-NL1C and ligated into pBS-NL1A at the corresponding sites, resulting in plasmid pBS-NL1B. For expression studies, a BamHIIApal fragment generated out of pBS-NL1B, corresponding to the full length cDNA of NL1, was inserted into pCDNA**3**/RSV [18] vector.

Production of polyclonal antibodies

A plasmid for the production in *Escherichia coli* of a GST fusion protein with NL1 was constructed using pGEX-4T-3 expression vector (Pharmacia Biotechnologies). A 255 bp fragment from NL1 was amplified by PCR with Vent polymerase using sense primer 5'-GCTACG GGATCCGTGGCCACTATGCTTAGGAA-3' (nt 1139 to 1158) and antisense primer 5'-CGATTG CTCGAGTGGGMCAGCTCGACTTCCA-3' (nt 1377 to 1396). Both pGEX-4T-3 and the PCR product were digested with BamHI and XhoI and ligated. The recombinant protein was produced and purified according to the supplier's instructions. Five weeks old female balb/c mice were immunized at monthly intervals for 3 months with 20 μg of the

recombinant NL1 fragment in Freund's adjuvant and antisera were subsequently collected.

Cell culture and transfection

Human Embryonic Kidney (HEK 293) cells were maintained in DMEM medium containing 10% fetal bovine serum (FBS), and supplemented with penicillin at 60 μg/ml, streptomycin at 100 μg/ml and fongizone at 0.25 μg/ml. Transfections of cells with appropriate plasmids were performed by the calcium/phosphate-DNA co-precipitation method (Chang 1987). To establish permanent cell lines, G418 selection was initiated 48 h after the transfections at 400 −82 g/ml for 12 days and gradually decreased at 100 μg/ml.

LLC-PK, cells transfected with pRcCMV-sNEP were maintained as described previously (Lanctot 1995).

Immunoblot analysis

For immunoblot analysis, cells were incubated for 16 h in synthetic DMEM medium containing 2 mM sodium butyrate. Cellular proteins were solubilized as previously described (Dion 1995). Secreted proteins recovered in culture media were concentrated approximately 10 fold by ultrafiltration. Immunoblot analysis were performed using the NEN Renaissance kit with the polyclonal antibody specific to NL1 or the α1-antitrypsin inhibitor antibody (Calbiochem; LaJolla, Calif.) followed by the appropriate horseradish peroxidase-conjugated IgG (Vector Laboratories).

For the glycosylation studies, proteins were incubated with endoglycosidase H (endoH) or peptide:N-glycosidase (PNGaseF) as suggested by the distributor (NEB).

Enzymatic activity assays

NL1 activity was monitored and compared to sNEP activity using (Tyrosyl-[3,5-^{3}H])(D-Ala$_2$)-Leu$_5$-enkephalin (50 Ci/mmol) (Research Products International Inc.), as already described (Dion 1995; Devault 1988). K_m values were determined by the isotope-dilution method. The inhibitory effects of phosphoramidon and thiorphan were also assessed as previously described (Dion 1995).

HPLC analysis of the hydrolysis of Leu-enkephalin

Five μg of Leu$_5$-enkephalin were incubated at 37° C. for one hour in 50 mM MES, pH 6.5, with concentrated culture medium of HEK 293 cells expressing NL1 (~300 μg of total proteins) or LLC-PK$_1$ cells expressing sNEP (~30 μg of total proteins), in absence or presence of 0.1 mM phosphoramidon. Hydrolysis products were separated by reversed-phase HPLC as described previously [23]. Tyr-Gly-Gly and Phe-Leu were both identified by elution profiles of synthetic marker peptides.

Northern blot analysis

A mouse multiple tissue poly(A)$^+$ mRNA blot (Clontech) was hybridized with a [^{32}P]dCTP random primer labelled probe in ExpressHyb solution (Clontech). The blot was washed according to the manufacturer's recommendations and exposed to Fuji RX film for 7 days at −80° C. with intensifying screens.

RT-PCR screening of mouse tissues

First strand cDNA synthesis was performed with 1 μg of total RNA from mouse tissues and oligo(dT) as primer, using Gene Amp RNA PCR Core Kit (Perkin Elmer). For the PCR reactions, primers 5'-TGGCGAGAGTGTGTCAGCTATGTC-3' and 5'-CTTCCAAAATGTAGTCAGGGTAGCCAATC-3' were used with Taq polymerase. One tenth of the PCR products were visualized on a 4% agarose gel.

In situ hybridization

To construct a plasmid for the synthesis of cRNA probes for ISH, pCR-NL1A was used as template to amplify a 452 bp fragment by PCR with sense primer 5'-GGAGCCATAGTGACTCTGGGTGTC-3' (nt 416 to 439) and antisense primer 5'-GACGCTCAGCAGGGGCTCAGAGTC-3' (nt 842 to 865). The amplification product was inserted into pCRII vector (Invitrogen). Synthesis of riboprobes and protocols for ISH were as described previously (Ruchon 1998).

Results

Cloning and sequence analysis of mouse NL1 cDNA

In order to isolate cDNAs for new members of the NEP family, we developed an RT-PCR strategy based on fact that NEP, ECE-1 and PHEX share regions of significant sequence identity. Following RT-PCR on testis mRNAs with nested primers, a DNA fragment of approximately **300** bp was amplified. This DNA fragment was cloned and the plasmids from 24 independent colonies were sequenced: 3 clones had no insert, 4 clones had DNA fragments not related to the NEP family, 7 clones had sequences corresponding to mouse NEP and 3 clones had sequences corresponding to mouse PHEX, showing that our approach efficiently allowed the identification of members of the family. Moreover, 7 identical clones had a new cDNA presenting sequence similarities to members of the NEP family. The full-length cDNA was subsequently obtained by screening a mouse testis A cDNA library followed by 5'RACE, as described under Materials and Methods. Its nucleotide and deduced amino acid sequences confirm that we cloned a novel NEP-like protein, referred to thereafter as NL1.

NL1 cDNA spans 2925 nt, including a 5'-untranslated region of 331 nt, an open reading frame of 2295 nt from nt 332 to nt 2626, and a 3'-untranslated region of 299 nt. The sequence surrounding the proposed initiator ATG conforms to the Kozak consensus (Kozak 1986). The deduced amino acid sequence of NL1 reveals a putative type **11** transmembrane protein of 765 amino acid residues encompassing a short N-terminal cytoplasmic tail, a unique putative transmembrane domain, and a large C-terminal extracellular domain. The ectodomain contains nine potential N-glycosylation sites (Asn-X-Ser/Thr) and ten cysteine residues corresponding to those conserved among all the members of the family, which are presumably involved in proper folding and in maintenance of the protein in an active conformation. All amino acid residues known to be part of the active site of NEP are present in NL1. The predicted protein presents greater similarities to NEP than to any other member of the family. Although NL1 shares numerous features with proteins of the neprilysin family, a notable aspect distinguishes it from the others: the first conserved cysteine residue of the ectodomain is more distant (34 amino acid residues) from the predicted transmembrane domain in NL1 than it is in NEP (9 residues) or any other members of the family. Moreover, we noticed a putative furin cleavage site (-Arg$_{58}$-Thr-Val-Val-Lys-Arg$_{63}$-) between the end of the transmembrane domain and the first cysteine. This observation suggests that NL1 could exist as a secreted rather than a membrane-bound protein.

NL1 expression in HEK 293 cells

HEK 293 cells were transfected with pCDNA**3**/RSV expression vector containing NL1 cDNA, and a permanent cell line was established as described under *Materials and Methods* (HEK/NL1 cells). Immunoblotting with a polyclonal antibody showed that after 16 h of culture, most NL1

was present in the culture medium with small amounts of the enzyme in the cell extract. Secreted and cell-associated NL1 had apparent molecular masses of approximately 125 and 110 kDa, respectively. To characterize the glycosylation state of NL1, we next submitted the recombinant protein to deglycosylation by peptide:N-glycosidase F (PNGase F) and endoglycosidase H (endo H). PNGase F removes high mannose as well as most complex N-linked oligosaccharides added in the Golgi complex. In contrast, endo H removes N-linked oligosaccharide side chains of the high mannose type found on proteins in the RER but which have not yet transited through the Golgi complex; thus, resistance to endo H can be used as an indication that the protein has traveled through the Golgi complex. PNGase F treatment showed that the cell-associated and secreted NL1 were N-glycosylated as their electrophoretic mobility increased following digestion. However, the secreted NL1 migrated as a doublet after PNGase F treatment, with one band co-migrating with cell-associated form and the second having a slower rate of migration. Since untreated and endo H-digested secreted NL1 are seen as single bands by SDS-PAGE, our observation suggests that a proportion of secreted NL1 undergoes further post-RER postranslational modification that renders some of the N-linked oligosaccharides resistant to PNGase F digestion.

In contrast to secreted NL1, NL1 from cell extract was sensitive to endo H treatment. This result shows differences in the glycosylation state of the two species and suggests that the cell-associated form observed in transfected cells is an intracellular species that has not traveled through the Golgi complex.

Processing of NL1 by a subtilisin-like convertase

To determine whether a member of the mammalian subtilisine-like convertase family is responsible for NL1 presence in the culture medium of transfected cells, we co-transfected transiently HEK 293 cells with a constant amount of plasmid pCDNA3/RSV/NL1 and increasing amounts of plasmid pCDNA3/CMV/PDX (Benjannet 1997). This latter vector promotes the expression of the α1-antitrypsin Portland variant, α1-PDX, a known inhibitor of subtilisin-like convertases (Anderson 1993). Immunoblot analysis of the culture media of cells expressing both NL1 and α1-PDX indicated that NL1 secretion was strongly inhibited by the presence of α1-PDX: a relation was observed between the amounts of α1-PDX and the level of inhibition of NL1 secretion.

To confirm that proteolysis by the subtilisin-like convertase occurred at the putative furin cleavage site identified in NL1 ectodomain (-Arg_{58}-Thr-Val-Val-Lys-Arg_{63}-), the amino acid residues Asn_{62}-Gly_{63} were substituted for Lys_{62}-Arg_{63} by site-directed mutagenesis in vector pCDNA3/RSV/NL1 and the mutated vector used to establish HEK 293 cells expressing the mutant protein (HEK/NL1mut cells). Immunoblot analysis of the culture media of HEK/NL1 mut cells showed that the mutation totally abolished secretion of NL1. Furthermore, an additional form of NL1 with a molecular mass of 127 kDa was detected in the extract of these cells. This new species was resistant to endo H digestion and was found associated with membranes when HEK/NL1mut cells were fractionated according to Chidiac et al. 1996 (result not shown).

NL1 enzymatic activity

Culture media from HEK 293 and HEK/NL1 cells were tested for enzymatic activity using as substrate (Tyrosyl-[3, 5-^{3}H](D-Ala_2)-Leu_5-enkephalin, a known NEP substrate. Activity was detected in the culture medium of HEK/NL1 cells but not in that of HEK 293 cells. This activity increased linearly with the amounts of NL1 and with the incubation period, indicating that degradation of the substrate was due to NL1 enzymatic activity.

We next characterized NL1 enzymatic parameters using the same substrate and compared them to those of an engineered soluble form of NEP (sNEP) (Lemay 1989). NL1 affinity for D-Ala_2-Leu_5-enkephalin was slightly higher than that of sNEP as shown by their K_m values of 18 μM and 73 μM, respectively. Inhibition assays showed that phosphoramidon had similar effects on NL1 and sNEP activity, with IC_{50} values of 0.9 nM and 0.5 nM respectively, and that thiorphan, a specific inhibitor of NEP, inhibited NL1 with an IC_{50} of 47 nM, as compared with an IC_{50} of 8 nM for NEP.

Very low levels of phosphoramidon-sensitive activity was detected in extracts of HEK/NL1 cells (data not shown) consistent with the small amounts of NL1 observed by immunoblotting.

To determine whether NL1 had cleavage site specificity similar to NEP, we incubated Leu_5-enkephalin in the presence of NL1 recovered from the medium of HEK/NL1 cells or in the presence of sNEP, and analyzed the degradation products by RP-HPLC. Peaks co-migrating with standard Tyr-Gly-Gly and Phe-Leu peptides were observed in both RP-HPLC profiles, indicating that both enzymes cleaved the substrate at the Gly_3-Phe_4 peptide bond. This enkephalin-degrading activity was completely inhibited by phosphoramidon (1 μM).

Tissue and cellular distribution of NL1 mRNA

Tissue distribution of NL1 mRNA was determined by Northern blot analysis with a specific probe corresponding to the 5'end of the coding region of NL1 cDNA. A single transcript of 3.4 kb was detected exclusively in testis among all the mouse tissues tested. Mouse tissues were also screened by RT-PCR. Using this more sensitive technique, expression of NL1 was observed in several other tissues including heart, brain, spleen, lungs, liver and kidney. Consistent with the Northern blot results, RT-PCR analysis, although not strictly quantitative, detected more NL1 mRNA in testis than in other tissues.

To gain more insight into NL1 mRNA distribution, we examined by ISH cryostat sagital sections from a 4-day newborn mouse, as well as sections from a 16-day old animal (p16) and adult tissues (heart, brain, spleen, lungs, liver, kidney and testis). The presence of NL1 mRNA was detected only in adult testis. Only the germinal cells in the luminal face of the seminiferous tubules were labeled. These cells were identified as round and elongated spermatids in all spermiogenesis maturational stages. Neither spermatozoa nor spermatocytes, spermatogonies or Sertoli cells were labeled. Interstitial cells were also negative. Controls were performed with sense riboprobes, which produced only nonspecific background (data not shown). The 4-day old mouse sagital sections and all other tissues tested were negative.

Discussion

The great interest in members of the Neprilysin family as putative therapeutic targets, and the recent discovery of new members of this important family of peptidases led us to investigate whether additional members of the family remained to be identified. Using a PCR-based strategy, we cloned, from mouse testis, a partial cDNA encoding a new NEP-like enzyme that we called NL1. Analysis of the amino acid sequence encoded by the full-length NL1 cDNA revealed that this member of the family resembles NEP the most: 55% identity and 74% similarity. Recently, the pri-

mary structure of a new zinc metallopeptidase from total mouse embryo was reported (Ikeda 1999). This enzyme, called SEP, is found either as a soluble or a cell-associated form due to alternative splicing. NL1 shows only 3 amino acid differences with the soluble form of SEP indicating that secreted SEP and NL1 are the same enzyme. Our cloning strategy did not allow characterization of the cell-associated form of NL1 which is a minor species in mouse testis (Ikeda 1999).

The amino acid sequence of NL1 predicts a topology of a type II integral membrane glycoprotein that is similar to the other members of the family. Treatment of the recombinant protein with PNGase F showed that indeed NL1 possesses N-linked carbohydrate side chains. However, it is not possible to determine precisely whether all nine putative N-glycosylation sites are used, but the 30 kDa decrease in molecular mass upon PNGase F treatment suggests that most are glycosylated. It has already been shown that all asparagine residues in a Asn-X-Ser/Thr consensus are glycosylated in rabbit NEP expressed in COS-1 cells and that sugar moieties increase the stability and enzymatic activity of the protein and facilitate its intracellular transport (Lafrance 1994). Three of NEP glycosylated Asn residues (Asn 145, Asn 285 and Asn 628) are conserved in NL1 (Asn 163, Asn 303 and Asn 643). Amongst these residues, Asn 145 and Asn 628 have been reported to influence NEP enzymatic activity (Lafrance 1994). In the same work, it has also been shown that the effect of sugar addition on folding and intracellular transport of NEP is a cumulative effect of all glycosylation sites rather than a contribution of any particular one. Glycosylation of NL1 may share similarities with that of NEP since we found their primary structures and enzymatic activities to be very similar.

Surprisingly, expression of the cDNA by transfection of HEK 293 cells showed that most of the enzyme was secreted in the culture medium. The small amount of NL1 associated with the cells was endo H-sensitive, suggesting that the cell-associated enzyme is a species that has not yet left the RER. The presence of a furin cleavage site in NL1 sequence between the predicted transmembrane domain and the first conserved cysteine residue of the ectodomain led us to believe that a member of the mammalian subtilisin-like family of convertases was responsible for the presence of NL1 in the culture medium. These enzymes are involved in processing a variety of precursor proteins such as growth factors and hormones, receptors, plasma proteins, matrix metalloproteinases, metalloproteases-desintegrins and viral envelope glycoproteins [for a review see: (Nakayama 1997). Site-directed mutagenesis of the furin cleavage site (-Arg_{58}-Thr-Val-Val-Lys-Arg_{63}-) and expression of α1-PDX, a potent inhibitor of mammalian subtilisin-like convertases (Anderson 1993), confirmed that a member of this family of endoproteases was involved in NL1 secretion presumably by cleaving in carboxy-terminus of Arg_{63}. There are only a few examples of proteins which are processed from a membrane-bound precursor to a secreted form following cleavage by subtilisin-like convertases; these include meprin and collagen XVII (Milhiet 1995; Schacke 1998). Three members of the subtilisin-like family of convertases, namely furin, PC4 and PC7, are known to be expressed in germ cells (Nakayama 1992; Torri 1993; Seidah 1992, 1996). Whether one of these convertases generates secreted NL1 from its membrane form is under current investigation. In any case, NL1 is the only known member of the neprilysin family that is secreted. This unique feature suggests that NL1 plays its physiological role in a context different from that of the membrane-bound peptidases, thereby diversifying the role of the peptidases of the neprilysin family. It is of interest that circulating forms of NEP in blood and urine have been described, but they have generally been related to pathological or stressful conditions (Almenoff 1984; Deschodt-Lanckmann 1989; Johnson 1985; Soleilhac 1996; Aviv 1995).

We have observed in cells expressing NL1 mutated at the furin cleavage site the appearance of a species resistant to digestion by endo H. This mutated protein was associated with cellular membranes. Taken together, these results indicate that NL1 is first synthesized and inserted in the RER membrane as a type II transmembrane protein. During intracellular transport, NL1 is converted to a soluble form by the action of a member of the mammalian subtilisin-like convertases. The identity of the cellular compartment where this process occurs is not known. However, mammalian subtilisin-like convertases are usually active in post-Golgi compartments of the secretory pathway suggesting that processing of NL1 from the membrane bound form to the soluble form is a post-Golgi event.

Despite almost total abrogation of NL1 secretion, we observed only a slight accumulation of endo H-resistant NL1 in cells either co-expressing α1-PDX and NL1 (result not shown) or expressing mutated NL1. This observation suggests that unprocessed NL1 is rapidly degraded. A similar behavior was reported for the Notch1 receptor expressed in the furin-deficient cell line LoVo (Logeat 1998). The mechanism(s) by which these unprocessed proteins are degraded is still unknown. It is interesting to point out that the spliceoform of SEP that has lost a 23 amino acid peptide, including the furin cleavage site, generates a cell-associated endo H-sensitive molecule (Ikeda 1999).

The most important observation regarding the NL1 primary structure is the conservation of residues which in NEP are essential for catalysis and binding of substrates or inhibitors. This finding suggests that NL1 could effectively act as an endopeptidase with a catalytic mechanism similar to that of NEP. This hypothesis was supported by the demonstration that D-Ala_2-Leu_5-enkephalin, a peptide substrate often used to monitor NEP activity, was also an excellent NL1 substrate. The affinity of NL1 for D-Ala_2-Leu_5-enkephalin was even higher than that of NEP, as reflected by a K_m value 4- to 5-fold lower. Furthermore, two well known NEP inhibitors, phosphoramidon and thiorphan, also abolished NL1 activity. Phosphoramidon, which inhibits NEP as well as ECE-1 activity, albeit to a lesser extent (Turner 1996), had very similar effects on NL1 and NEP, with an IC_{50} value for NL1 varying not more than two-fold from the value determined for NEP. Thiorphan, considered to be a more specific inhibitor of NEP, also inhibited NL1 activity, with an IC_{50} six-fold greater than that for NEP. These results suggest that the active sites of NL1 and NEP are similar. This hypothesis is supported by the observation that secreted SEP degraded a set of peptides known to be NEP substrates, including substance P, bradykinin and atrial natiuretic peptide (Ikeda 1999). Taken together, these results illustrate the importance of identifying and characterizing other member of the family for the design of highly specific inhibitors.

In agreement with the enzymatic parameters demonstrating that NL1 and NEP have similar catalytic sites, we have observed that both enzymes cleaved Leu_5-enkephalin at the same peptide bond. This result suggests that NL1 hydrolyzes peptide bonds on the amino side of hydrophobic amino acid residues as does NEP (Turner 1985). However, several other peptides will have to be tested to confirm this specificity and to determine whether NL1 has dipeptidyl carboxypeptidase

activity as was shown for NEP (Malfroy 1982; Bateman 1989; Beaumont 1991) and more recently for ECE-1 (Johnson 1999).

RT-PCR experiments with specific primers for the soluble and cell-associated forms of SEP showed a wide tissue distribution of the enzyme with the soluble form of SEP being predominant in testis and the cell-associated form in other tissues (Ikeda 1999). Our RT-PCR results confirmed the wide tissue distribution of NL1. However, Northern blotting and in situ hybridization experiments indicated that expression of NL1 is predominant in germ cells of mature testis. Interestingly, proenkephalin mRNA has been shown to be expressed in germ cells and somatic cells of the testis (Torii 1993, Seidah 1992; Kew 1989; Mehta 1994; Kilpatrick 1986, 1987). Specific functions for testicular enkephalin peptides have not yet been defined, but it is believed that they could act as intratesticular paracrine/autocrine factors. In addition to their putative role as mediators of testicular cell communication, it has also been demonstrated that proenkephalin products synthesized by spermatogenic cells during spermatogenesis are stored in the acrosome of human, hamster, rat and sheep spermatozoa and are release from sperm following acrosomal reaction (Kew 1990). It has thus been proposed that proenkephalin products may act as sperm acrosomal factors during the fertilization process as well as intratesticular regulators secreted by spermatogenic cells. Since Leu_5-enkephalin was found to be a good substrate for NL1, opioid peptides originating from proenkephalin could serve as physiological substrate for this new enzyme. In this way, NL1 would serve to regulate the activity of these bioactive peptides.

Testis is the only tissue where the soluble form of SEP is predominant (Ikeda, 1999), suggesting a testis-specific alternative splicing. Expression of testis-specific molecular species of peptidases or prohormones, arising through diverse mechanisms, has been documented in the past (Howard 1990; Jeannotte 1987). However, the physiological significance of these testis-specific species is not always clear. In the case of NL1 or SEP, it might allow local constitutive secretion by germinal cells of an otherwise cell-associated enzyme, to regulate spermatogenesis much like several other proteolytic enzymes of the seminiferous tubules (Monsees 1998). Alternatively, it might allow accumulation in acrosome with proenkephalin peptides and release upon acrosomal reaction. More exhaustive studies concerning NL1 localization and physiological substrate identification will be needed to understand its role in the testis and possibly in the fertilization process.

Cloning of other members of the family

To find other members of the NEP-like family, we will use the same RT-PCR strategy to amplify mRNA isolated from tissues known to be regulated by peptidergic systems (brain, thymus, kidney, heart, lung, ovary, pancreas, bone, bone marrow and lymphoid cells). In fact, many of these tissues are known to express at least one member of the family and/or to control a peptidergic pathway on which peptidase inhibitors have major effects. Amplified fragments will be cloned and the resulting clones will be sequenced and compared to the sequence of known peptidases, as described above. Pairs of degenerate primers in other highly conserved regions will also be designed to increase the possibility of cloning other relevant peptidases.

DISCUSSION

As discussed above, peptidases of the NEP family known to date have often been found to play important physiological roles. This is certainly true for NEP itself, ECEs and PEX, (see review above). For this reason, some of these enzymes (as it was the case for NEP and ECE in the past) might be interesting targets for the design of inhibitors that in turn could be used as therapeutic agents in various pathological conditions. However, it is of some concern that inhibitors designed for one enzyme may also inhibit to some extent other members of the family. This lack of specificity for an inhibitor used as a therapeutic agent in the long term treatments such as those used as antihypertensive agents for instance, may cause unforeseen problems due to unwanted side effects. The objectives of the present work was to develop a strategy to clone new members of the NEP family of peptidases. The results presented in this report clearly show that our strategy can be successful. We have determined the complete or partial nucleotide sequence of three cDNAs encoding putative enzymes of the NEP family.

These cDNA sequences are valuable tools and may be used to:

Produce antibodies

As shown in the present work, knowledge of NL cDNA sequences can be used to raise specific antibodies. For example but not exclusively, regions of less homology between the peptidases (amino acid residues 50 to 450) can be used to synthesize peptides whose sequences are deduced from the translation of the cDNAs, and/or bacterially-expressed fragments of the cDNAs fused for example but not exclusively to GST may be purified and injected into rabbits or mice for polyclonal or monoclonal antibody production. These antibodies can be used to:

identify by immunohistochemistry the peptidergic pathways in which the peptidases are functioning;

study the physiopathology of NL-enzymes by immunoblotting or immunohistochemistry on samples of biological fluids or biopsies;

set up high through put screening assays to identify NL-enzymes inhibitors. This can be done for example but not exclusively by using the antibodies to attach the NL-enzymes to a solid support;

purify NL-enzymes with said antibodies by immunoprecipitation or affinity chromatography by identifying antibodies capable of selectively binding to the NL-enzymes in one set of conditions and releasing it in another set of conditions typically involving a large pH or salt concentration change without denaturing the NL-enzyme;

identify antibodies that block NL-enzymes activities and use them as therapeutic agents. Blocking antibodies can be identified by adding antisera or ascite fluid to an in vitro enzymatic assay and looking for inhibition of NL-enzymes activities. Blocking antibodies could then be injected to normal or disease model animals to test for in vivo effects.

Derive specific RNA or DNA probes

As shown in the present work, knowledge of the nucleotide sequence of the members of the NEP-family allows nucleotide sequence comparisons and facilitate the design of specific RNA or DNA probes by methods such as but not exclusively molecular cloning, in vitro transcription, PCR or DNA synthesis. The probes thus obtained can be used to:

derive specific probes or oligonucleotides for RNA and DNA analysis, such as Northern blot and in situ hybridization, chromosome mapping or PCR testing.

These probes could be used for genetic testing of normal or pathological samples of biological fluids or biopsies;

make vectors for gene knock-out or knock-in in mice. The long range PCR technique and/or screening of a mouse genomic library with probes derived from the 5'-end of the cDNAs can be used to isolate large exon/intron regions. We will then substitute one or more of the cloned genomic DNA exons for the neomycin resistance expression cassette for producing homologous recombination and knock-out mice. Alternatively, cDNAs coding for NLs will be used to overexpressed each of these enzymes in transgenic mice. The cDNAs will be cloned downstream from a promoter sequence, and injected in fertilised mouse eggs. Depending on specific questions to be answered, the chosen promoter sequence will allow expression of the peptidases either in every tissues or in a cell- or tissue-specific manner. Injected eggs will be transferred into foster mothers and the resulting mice analysed for peptidase expression;

replace defective NL genes in a gene therapy strategy. The NL full length cDNAs could be cloned under the control of a constitutive or inducible promoter having a narrow or wide range of tissue expression and introduced with appropriate vectors in subjects having defective genes;

synthesise oligonucleotides that could be used to interfere with the expression of the NLs. For example but not exclusively, oligonucleotides with antisens or ribozyme activity could be developed. These oligonucleotides could be introduced in subjects as described above;

isolate other members of the family. Screening cDNA and/or genomic libraries with these cDNA probes at low stringency may allow to clone new members of the NEP-like family. Alternatively, alignment of the sequences may allow one to design specific degenerate oligonucleotide primers for RT-PCR screening with mRNA from tissues such as but not exclusively, the hearth and the brain.

Production of recombinant NL-enzymes

As shown in the present work, recombinant active NL-enzymes can be obtained by expression of NL-cDNAs in mammalian cells. From past experience with neprilysin, another member of the family (Devault et al., 1988; Fossiez et al., 1992; Ellefsen et al., submitted), expression can also be performed in other expression systems after cloning of NL-cDNAs in appropriate expression vectors. These expression systems may include but not exclusively the baculovirus/insect cells or larvae system and the *Pichia pastoris*-based yeast system. Production of recombinant NL-enzymes includes the production of naturally occurring membrane bound or soluble forms of the proteins or genetically engineered soluble forms of the enzymes. The latter can be obtained by substituting the cytosolic and trans-membrane domain by a cleavable signal peptide such as that of proopiomelanocortin, but not exclusively, as done previously (Lemay et al., 1989) or by transforming by genetic manipulations the non-cleavable signal peptide membrane anchor domain into a cleavable signal peptide, as done previously (Lemire et al., 1997) or by fusion of the ectodomain of NL-enzymes to the amino-terminal domain (from the initiator methionine to amino acid residue **300**) of naturally occurring soluble NLs such as, but not exclusively, NL-1 as done in this work. These recombinant NLs could be used to:

find a substrate. A substrate can be identified using one of the following.

Screening of existing bioactive peptides. Peptides are incubated in the presence of NL-enzymes and subsequently analysed by HPLC for degradation. Degradation is observed by disappearance of the peak of substrate and the appearance of peaks of products;

Screening phage libraries specifically designed for the purpose (phage display library). Each phage expresses at its surface, as part of its coat protein, a random peptide sequence preceded by a peptide sequence recognisable by an antibody or any other sequence-recognizing agent. This latter sequence serves to attach the phage to a solid support. Upon addition of the NL-enzyme the random sequences that are NL substrate are cleaved, releasing the phage. After several rounds of cleavage, the phage sequence is determined to identify the peptide segment recognized by the enzyme.

Extract of the tissue where the enzyme is expressed is collected and prepared for chromatographic analysis (HPLC, capillary electrophoresis or any other high resolution separation system) by denaturing the extracted proteins with a solvent (acetonitrile or methanol). The extract is subjected to chromatographic separation. The same extract is incubated with the enzyme for a period sufficient to observe a difference between the 2 chromatograms. The regions with the identified changes are collected and subjected to mass spectrometric analysis to determine the peptide compositions.

Small peptide libraries are prepared with a fluorophore at one extremity and a quencher group at the other (Meldal et al Methods in molecular biology 1998, 87). The substrate can be identified using a strategy described in Apletalina et al (JBC (1998)273, 41, 26589–95). For each hexapeptide library, the identity of one residue at one position remains constant while the rest is randomized (for a total of 6*20=120 individual libraries). Each library is made-up of 3.2 million different members and is identified both by the position of the constant residue along the hexapeptide, and its identity. The NL-enzyme is added to each library and the fluorescence is recorded. The data is organized to identify the libraries producing the most fluorescence for each position along the hexapeptide. This arrangement suggests the identity of important residues at each position along the hexapeptide. Hexapeptide representing the best suggestions are prepared and tested in a similar fashion. From this set, the hexapeptide with the best fluorescence is selected.

set up enzymatic assays. An enzymatic assay consists in the addition of the above-identified substrate to the enzyme in constant conditions of pH, salts, temperature and time. The resulting solution is assayed for the hydrolysed peptide or for the intact peptide. This assay can be realized with specific antibodies, HPLC or, when self-quenched fluorescence tagged peptides are used (Meldal et al), by the appearance of fluorescence. The enzyme may be in solution or attached to a solid substrate;

identify inhibitors. Inhibitors can be identified from synthetic libraries, biota extracts and from rationally designed inhibitors using X-ray crystallography and

substituent activity relationships. Each molecule or extract fraction is tested for inhibitory activity using the enzymatic test described above. The molecule responsible for the largest inhibition is further tested to determine its pharmacological and toxicological properties following known procedures. The inhibitor with the best distribution, pharmacological action combined with low toxicity will be selected for drug manufacturing. Pharmaceutically acceptable formulation of the inhibitor or its acceptable salt will be prepared by mixing with known excipients to produce tablets, capsules or injectable solutions. Between 1 and 500 mg of the drug is administered to the patients;

inject the native or soluble purified NL-enzymes into subjects. In the case of disease or pathologies caused by a lack or decrease in NL activity, the purified NL could be injected intravenously or otherwise in patients. Alternatively, immobilized NL-enzymes could be introduced at the site of orthopedic surgery or implantation of devices in bones or dental tissues.

Secretion of foreign proteins and peptides

As shown in the present work, the amino-terminal domain of NL-1 (from the initiator methionine to the furin site) can be used to promote the secretion of a foreign protein (in this case the ectodomain of NL-3 and β-endorphin).

The amino-terminal domain of NL-1 but also of other naturally occurring soluble

NL-enzymes could be used to:

promote production and secretion of foreign proteins. This can be achieved by genetically fusing sequences coding for said foreign proteins downstream from and in phase with the amino-terminal of NL-1. These chimeric constructs could be introduced with the help of appropriate vectors in any of the expression systems mentioned above for protein production and secretion;

promote production and secretion of bioactive peptides. Sequences encoding small bioactive peptides such as but not exclusively β-endorphin, the enkephalins, substance P, atrial natriuretic peptide (ANF) and osteostatine, could be fused immediately downstream and in phase the furin site of NL-1. These DNA constructs could be used as described above to produce bioactive peptides.

serve as model to design artificial (non-naturally occurring) proteins or protein segments (protein vectors) to promote secretion of proteins or peptides. These protein vectors can be constructed to resemble a secreted protein. In this case they would be assembled of an endoplasmic reticulum signal peptide, a spacer of varying length and a furin cleavage site to which the protein or peptide destined for secretion can be fused. The total length of the spacer, furin cleavage site and protein or peptide destined for secretion must be at least **70** amino acid residues. Alternatively, such protein vectors could be assembled to resemble a type II membrane protein. In this case they would comprise from the amino to the carboxy-terminus a cytosolic domain of varying length, a transmembrane domain that also acts as a signal peptide, an extracellular segment of varying length and a furin cleavage site to which the protein or peptide destined for secretion can be fused. The total length of the extracellular segment, furin cleavage site and protein or peptide destined for secretion must be at least 70 amino acid residues.

Therapeutic applications of NL-enzymes

The inappropriate processing of endogenous peptides causes several diseases. The inappropriate processing may result from pathologic concentration of the enzyme itself, its substrate or other elements of the biochemical machinery downstream from the controlling enzyme. In this context it is possible to help the patient by managing the activity of the controlling enzyme.

NL-enzymes have been localized to the brain and may be involved in the improper processing of β-amyloid precursor. Inhibitions of this process by drugs prepared as above, will help patients with Alzheimer disease as well as other patient suffering from diseases caused by plaque formation;

NL-enzymes may be involved in the improper processing of other peptides involved in neurological diseases, pain or psychiatric disorders. Appropriately designed inhibitors will help in the management of such diseases;

NL-1 is found in testis and is associated with spermatozoid maturation. Peptides improperly processed by the enzyme may lead to infertility. The addition of NL-1 ex-vivo to seminal liquid or immature spermatozoids taken directly from testis during an in-vitro fertilization procedure will increase fertility. Conversely, the use of a small-molecule inhibitor or removal of NL-1 with an antibody could increase fertility during an in-vitro fertilization procedure. The administration of a NL-1 inhibitor may increase or decrease the fertility potential. This inhibitor is formulated and administered as described above.

NL-3 is found in ovaries and may be involved in the processing of a peptide involved in the maturation of eggs. The addition of NL-3 ex-vivo to immature eggs taken directly from ovaries during an in-vitro fertilization procedure will increase fertility. Conversely, the use of a small-molecule inhibitor or removal of NL-3 with an antibody could increase fertility during an in-vitro fertilization procedure. This inhibitor is formulated and administered as described above;

NL-3 is found in bones. The improper processing of peptides by the enzyme may result in bone disease or abnormal phosphate metabolism. Administration of an inhibitor, as described above, will allow the disease management.

TABLE I

Extend of amino acid sequence identity between members of the NEP-like family

	hNEP	hPEX	hECE-1A	hECE-2	hKELL	sNL-1	hNL-2	hNL-3
hNEP	100*							
hPEX	35	100						
hECE-1A	39	38	100					
hECE-2	36	37	62	100				
hKELL	23	24	30	31	100			
sNL-1	55	39	39	39	26	100		
hNL-2	54	39	39	39	26	77	100	
hNL-3	35	32	37	37	28	36	34	100

*percentage of sequence identity

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Lys Pro Glu Asp Arg Asn Asp Pro Met Leu Leu Tyr Asn Lys Met Thr
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Ser Ile Thr Asn Glu Glu Asp Val Val Val Tyr Ala Pro Glu Tyr Leu
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Gln Gln Ser Ala Ser Asn Phe Lys Glu Gln Ser Gln Cys Met Val Tyr
    610                 615                 620

Gln Tyr Gly Asn Phe Ser Trp Asp Leu Ala Gly Gly Gln His Leu Asn
625                 630                 635                 640

Gly Ile Asn Thr Leu Gly Glu Asn Ile Ala Asp Asn Gly Gly Leu Gly
                645                 650                 655

Gln Ala Tyr Arg Ala Tyr Gln Asn Tyr Ile Lys Lys Asn Gly Glu Glu
            660                 665                 670

Lys Leu Leu Pro Gly Leu Asp Leu Asn His Lys Gln Leu Phe Phe Leu
        675                 680                 685

Asn Phe Ala Gln Val Trp Cys Gly Thr Tyr Arg Pro Glu Tyr Ala Val
    690                 695                 700

Asn Ser Ile Lys Thr Asp Val His Ser Pro Gly Asn Phe Arg Ile Ile
705                 710                 715                 720

Gly Thr Leu Gln Asn Ser Ala Glu Phe Ser Glu Ala Phe His Cys Arg
                725                 730                 735

Lys Asn Ser Tyr Met Asn Pro Glu Lys Lys Cys Arg Val Trp
            740                 745                 750


<210> SEQ ID NO 2
<211> LENGTH: 749
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2

Met Glu Ala Glu Thr Gly Ser Ser Val Glu Thr Gly Lys Lys Ala Asn
  1               5                  10                  15

Arg Gly Thr Arg Ile Ala Leu Val Val Phe Val Gly Gly Thr Leu Val
             20                  25                  30

Leu Gly Thr Ile Leu Phe Leu Val Ser Gln Gly Leu Leu Ser Leu Gln
         35                  40                  45

Ala Lys Gln Glu Tyr Cys Leu Lys Pro Glu Cys Ile Glu Ala Ala Ala
     50                  55                  60

Ala Ile Leu Ser Lys Val Asn Leu Ser Val Asp Pro Cys Asp Asn Phe
 65                  70                  75                  80

Phe Arg Phe Ala Cys Asp Gly Trp Ile Ser Asn Asn Pro Ile Pro Glu
                 85                  90                  95

Asp Met Pro Ser Tyr Gly Val Tyr Pro Trp Leu Arg His Asn Val Asp
            100                 105                 110

Leu Lys Leu Lys Glu Leu Leu Glu Lys Ser Ile Ser Arg Arg Arg Asp
        115                 120                 125

Thr Glu Ala Ile Gln Lys Ala Lys Ile Leu Tyr Ser Ser Cys Met Asn
    130                 135                 140

Glu Lys Ala Ile Glu Lys Ala Asp Ala Lys Pro Leu Leu His Ile Leu
145                 150                 155                 160

Arg His Ser Pro Phe Arg Trp Pro Val Leu Glu Ser Asn Ile Gly Pro
                165                 170                 175

Glu Gly Val Trp Ser Glu Arg Lys Phe Ser Leu Leu Gln Thr Leu Ala
            180                 185                 190

Thr Phe Arg Gly Gln Tyr Ser Asn Ser Val Phe Ile Arg Leu Tyr Val
        195                 200                 205

Ser Pro Asp Asp Lys Ala Ser Asn Glu His Ile Leu Lys Leu Asp Gln
    210                 215                 220

Ala Thr Leu Ser Leu Ala Val Arg Glu Asp Tyr Leu Asp Asn Ser Thr
225                 230                 235                 240
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Glu Ala Lys Ser Tyr Arg Asp Ala Leu Tyr Lys Phe Met Val Asp Thr
                245                 250                 255

Ala Val Leu Leu Gly Ala Asn Ser Ser Arg Ala Glu His Asp Met Lys
            260                 265                 270

Ser Val Leu Arg Leu Glu Ile Lys Ile Ala Glu Ile Met Ile Pro His
        275                 280                 285

Glu Asn Arg Thr Ser Glu Ala Met Tyr Asn Lys Met Asn Ile Ser Glu
    290                 295                 300

Leu Ser Ala Met Ile Pro Gln Phe Asp Trp Leu Gly Tyr Ile Lys Lys
305                 310                 315                 320

Val Ile Asp Thr Arg Leu Tyr Pro His Leu Lys Asp Ile Ser Pro Ser
                325                 330                 335

Glu Asn Val Val Val Arg Val Pro Gln Tyr Phe Lys Asp Leu Phe Arg
            340                 345                 350

Ile Leu Gly Ser Glu Arg Lys Lys Thr Ile Ala Asn Tyr Leu Val Trp
        355                 360                 365

Arg Met Val Tyr Ser Arg Ile Pro Asn Leu Ser Arg Arg Phe Gln Tyr
    370                 375                 380

Arg Trp Leu Glu Phe Ser Arg Val Ile Gln Gly Thr Thr Thr Leu Leu
385                 390                 395                 400

Pro Gln Trp Asp Lys Cys Val Asn Phe Ile Glu Ser Ala Leu Pro Tyr
                405                 410                 415

Val Val Gly Lys Met Phe Val Asp Val Tyr Phe Gln Glu Asp Lys Lys
            420                 425                 430

Glu Met Met Glu Glu Leu Val Glu Gly Val Arg Trp Ala Phe Ile Asp
        435                 440                 445

Met Leu Glu Lys Glu Asn Glu Trp Met Asp Ala Gly Thr Lys Arg Lys
    450                 455                 460

Ala Lys Glu Lys Ala Arg Ala Val Leu Ala Lys Val Gly Tyr Pro Glu
465                 470                 475                 480

Phe Ile Met Asn Asp Thr His Val Asn Glu Asp Leu Lys Ala Ile Lys
                485                 490                 495

Phe Ser Glu Ala Asp Tyr Phe Gly Asn Val Leu Gln Thr Arg Lys Tyr
            500                 505                 510

Leu Ala Gln Ser Asp Phe Phe Trp Leu Arg Lys Ala Val Pro Lys Thr
        515                 520                 525

Glu Trp Phe Thr Asn Pro Thr Thr Val Asn Ala Phe Tyr Ser Ala Ser
    530                 535                 540

Thr Asn Gln Ile Arg Phe Pro Ala Gly Glu Leu Gln Lys Pro Phe Phe
545                 550                 555                 560

Trp Gly Thr Glu Tyr Pro Arg Ser Leu Ser Tyr Gly Ala Ile Gly Val
                565                 570                 575

Ile Val Gly His Glu Phe Thr His Gly Phe Asp Asn Asn Gly Arg Lys
            580                 585                 590

Tyr Asp Lys Asn Gly Asn Leu Asp Pro Trp Trp Ser Thr Glu Ser Glu
        595                 600                 605

Glu Lys Phe Lys Glu Lys Thr Lys Cys Met Ile Asn Gln Tyr Ser Asn
    610                 615                 620

Tyr Tyr Trp Lys Lys Ala Gly Leu Asn Val Lys Gly Lys Arg Thr Leu
625                 630                 635                 640

Gly Glu Asn Ile Ala Asp Asn Gly Gly Leu Arg Glu Ala Phe Arg Ala
                645                 650                 655
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Tyr Arg Lys Trp Ile Asn Asp Arg Arg Gln Gly Leu Glu Glu Pro Leu
660 665 670

Leu Pro Gly Ile Thr Phe Thr Asn Asn Gln Leu Phe Phe Leu Ser Tyr
675 680 685

Ala His Val Arg Cys Asn Ser Tyr Arg Pro Glu Ala Ala Arg Glu Gln
690 695 700

Val Gln Ile Gly Ala His Ser Pro Pro Gln Phe Arg Val Asn Gly Ala
705 710 715 720

Ile Ser Asn Phe Glu Glu Phe Gln Lys Ala Phe Asn Cys Pro Pro Asn
725 730 735

Ser Thr Met Asn Arg Gly Met Asp Ser Cys Arg Leu Trp
740 745

<210> SEQ ID NO 3
<211> LENGTH: 732
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 3

Met Glu Gly Gly Asp Gln Ser Glu Glu Glu Pro Arg Glu Arg Ser Gln
1 5 10 15

Ala Gly Gly Met Gly Thr Leu Trp Ser Gln Glu Ser Thr Pro Glu Glu
20 25 30

Arg Leu Pro Val Glu Gly Ser Arg Pro Trp Ala Val Ala Arg Arg Val
35 40 45

Leu Thr Ala Ile Leu Ile Leu Gly Leu Leu Leu Cys Phe Ser Val Leu
50 55 60

Leu Phe Tyr Asn Phe Gln Asn Cys Gly Pro Arg Pro Cys Glu Thr Ser
65 70 75 80

Val Cys Leu Asp Leu Arg Asp His Tyr Leu Ala Ser Gly Asn Thr Ser
85 90 95

Val Ala Pro Cys Thr Asp Phe Phe Ser Phe Ala Cys Gly Arg Ala Lys
100 105 110

Glu Thr Asn Asn Ser Phe Gln Glu Leu Ala Thr Lys Asn Lys Asn Arg
115 120 125

Leu Arg Arg Ile Leu Glu Val Gln Asn Ser Trp His Pro Gly Ser Gly
130 135 140

Glu Glu Lys Ala Phe Gln Phe Tyr Asn Ser Cys Met Asp Thr Leu Ala
145 150 155 160

Ile Glu Ala Ala Gly Thr Gly Pro Leu Arg Gln Val Ile Glu Glu Leu
165 170 175

Gly Gly Trp Arg Ile Ser Gly Lys Trp Thr Ser Leu Asn Phe Asn Arg
180 185 190

Thr Leu Arg Leu Leu Met Ser Gln Tyr Gly His Phe Pro Phe Phe Arg
195 200 205

Ala Tyr Leu Gly Pro His Pro Ala Ser Pro His Thr Pro Val Ile Gln
210 215 220

Ile Asp Gln Pro Glu Phe Asp Val Pro Leu Lys Gln Asp Gln Glu Gln
225 230 235 240

Lys Ile Tyr Ala Gln Ile Phe Arg Glu Tyr Leu Thr Tyr Leu Asn Gln
245 250 255

Leu Gly Thr Leu Leu Gly Gly Asp Pro Ser Lys Val Gln Glu His Ser
260 265 270

Ser Leu Ser Ile Ser Ile Thr Ser Arg Leu Phe Gln Phe Leu Arg Pro
275 280 285

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Leu Glu Gln Arg Arg Ala Gln Gly Lys Leu Phe Gln Met Val Thr Ile
    290                 295                 300

Asp Gln Leu Lys Glu Met Ala Pro Ala Ile Asp Trp Leu Ser Cys Leu
305                 310                 315                 320

Gln Ala Thr Phe Thr Pro Met Ser Leu Ser Pro Ser Gln Ser Leu Val
                325                 330                 335

Val His Asp Val Glu Tyr Leu Lys Asn Met Ser Gln Leu Val Glu Glu
            340                 345                 350

Met Leu Leu Lys Gln Arg Asp Phe Leu Gln Ser His Met Ile Leu Gly
        355                 360                 365

Leu Val Val Thr Leu Ser Pro Ala Leu Asp Ser Gln Phe Gln Glu Ala
    370                 375                 380

Arg Arg Lys Leu Ser Gln Lys Leu Arg Glu Leu Thr Glu Gln Pro Pro
385                 390                 395                 400

Met Pro Ala Arg Pro Arg Trp Met Lys Cys Val Glu Glu Thr Gly Thr
                405                 410                 415

Phe Phe Glu Pro Thr Leu Ala Ala Leu Phe Val Arg Glu Ala Phe Gly
            420                 425                 430

Pro Ser Thr Arg Ser Ala Ala Met Lys Leu Phe Thr Ala Ile Arg Asp
        435                 440                 445

Ala Leu Ile Thr Arg Leu Arg Asn Leu Pro Trp Met Asn Glu Glu Thr
    450                 455                 460

Gln Asn Met Ala Gln Asp Lys Val Ala Gln Leu Gln Val Glu Met Gly
465                 470                 475                 480

Ala Ser Glu Trp Ala Leu Lys Pro Glu Leu Ala Arg Gln Glu Tyr Asn
                485                 490                 495

Asp Ile Gln Leu Gly Ser Ser Phe Leu Gln Ser Val Leu Ser Cys Val
            500                 505                 510

Arg Ser Leu Arg Ala Arg Ile Val Gln Ser Phe Leu Gln Pro His Pro
        515                 520                 525

Gln His Arg Trp Lys Val Ser Pro Trp Asp Val Asn Ala Tyr Tyr Ser
    530                 535                 540

Val Ser Asp His Val Val Val Phe Pro Ala Gly Leu Leu Gln Pro Pro
545                 550                 555                 560

Phe Phe His Pro Gly Tyr Pro Arg Ala Val Asn Phe Gly Ala Ala Gly
                565                 570                 575

Ser Ile Met Ala His Glu Leu Leu His Ile Phe Tyr Gln Leu Leu Leu
            580                 585                 590

Pro Gly Gly Cys Leu Ala Cys Asp Asn His Ala Leu Gln Glu Ala His
        595                 600                 605

Leu Cys Leu Lys Arg His Tyr Ala Ala Phe Pro Leu Pro Ser Arg Thr
    610                 615                 620

Ser Phe Asn Asp Ser Leu Thr Phe Leu Glu Asn Ala Ala Asp Val Gly
625                 630                 635                 640

Gly Leu Ala Ile Ala Leu Gln Ala Tyr Ser Lys Arg Leu Leu Arg His
                645                 650                 655

His Gly Glu Thr Val Leu Pro Ser Leu Asp Leu Ser Pro Gln Gln Ile
            660                 665                 670

Phe Phe Arg Ser Tyr Ala Gln Val Met Cys Arg Lys Pro Ser Pro Gln
        675                 680                 685

Asp Ser His Asp Thr His Ser Pro Pro His Leu Arg Val His Gly Pro
    690                 695                 700
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Leu Ser Ser Thr Pro Ala Phe Ala Arg Tyr Phe Arg Cys Ala Arg Gly
705                 710                 715                 720

Ala Leu Leu Asn Pro Ser Ser Arg Cys Gln Leu Trp
                725                 730


<210> SEQ ID NO 4
<211> LENGTH: 753
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

Met Ser Thr Tyr Lys Arg Ala Thr Leu Asp Glu Glu Asp Leu Val Asp
  1               5                  10                  15

Ser Leu Ser Glu Gly Asp Ala Tyr Pro Asn Gly Leu Gln Val Asn Phe
             20                  25                  30

His Ser Pro Arg Ser Gly Gln Arg Cys Trp Ala Ala Arg Thr Gln Val
         35                  40                  45

Glu Lys Arg Leu Val Val Leu Val Val Leu Leu Ala Ala Gly Leu Val
     50                  55                  60

Ala Cys Leu Ala Ala Leu Gly Ile Gln Tyr Gln Thr Arg Ser Pro Ser
 65                  70                  75                  80

Val Cys Leu Ser Glu Ala Cys Val Ser Val Thr Ser Ser Ile Leu Ser
                 85                  90                  95

Ser Met Asp Pro Thr Val Asp Pro Cys His Asp Phe Phe Ser Tyr Ala
            100                 105                 110

Cys Gly Gly Trp Ile Lys Ala Asn Pro Val Pro Asp Gly His Ser Arg
        115                 120                 125

Trp Gly Thr Phe Ser Asn Leu Trp Glu His Asn Gln Ala Ile Ile Lys
    130                 135                 140

His Leu Leu Glu Asn Ser Thr Ala Ser Val Ser Glu Ala Glu Arg Lys
145                 150                 155                 160

Ala Gln Val Tyr Tyr Arg Ala Cys Met Asn Glu Thr Arg Ile Glu Glu
                165                 170                 175

Leu Arg Ala Lys Pro Leu Met Glu Leu Ile Glu Arg Leu Gly Gly Trp
            180                 185                 190

Asn Ile Thr Gly Pro Trp Ala Lys Asp Asn Phe Gln Asp Thr Leu Gln
        195                 200                 205

Val Val Thr Ala His Tyr Arg Thr Ser Pro Phe Phe Ser Val Tyr Val
    210                 215                 220

Ser Ala Asp Ser Lys Asn Ser Asn Ser Asn Val Ile Gln Val Asp Gln
225                 230                 235                 240

Ser Gly Leu Gly Leu Pro Ser Arg Asp Tyr Tyr Leu Asn Lys Thr Glu
                245                 250                 255

Asn Glu Lys Val Leu Thr Gly Tyr Leu Asn Tyr Met Val Gln Leu Gly
            260                 265                 270

Lys Leu Leu Gly Gly Gly Asp Glu Glu Ala Ile Arg Pro Gln Met Gln
        275                 280                 285

Gln Ile Leu Asp Phe Glu Thr Ala Leu Ala Asn Ile Thr Ile Pro Gln
    290                 295                 300

Glu Lys Arg Arg Asp Glu Glu Leu Ile Tyr His Lys Val Thr Ala Ala
305                 310                 315                 320

Glu Leu Gln Thr Leu Ala Pro Ala Ile Asn Trp Leu Pro Phe Leu Asn
                325                 330                 335

Thr Ile Phe Tyr Pro Val Glu Ile Asn Glu Ser Glu Pro Ile Val Val
            340                 345                 350
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Tyr Asp Lys Glu Tyr Leu Glu Gln Ile Ser Thr Leu Ile Asn Thr Thr
        355                 360                 365

Asp Arg Cys Leu Leu Asn Asn Tyr Met Ile Trp Asn Leu Val Arg Lys
    370                 375                 380

Thr Ser Ser Phe Leu Asp Gln Arg Phe Gln Asp Ala Asp Glu Lys Phe
385                 390                 395                 400

Met Glu Val Met Tyr Gly Thr Lys Lys Thr Cys Leu Pro Arg Trp Lys
                405                 410                 415

Phe Cys Val Ser Asp Thr Glu Asn Asn Leu Gly Phe Ala Leu Gly Pro
            420                 425                 430

Met Phe Val Lys Ala Thr Phe Ala Glu Asp Ser Lys Ser Ile Ala Thr
        435                 440                 445

Glu Ile Ile Leu Glu Ile Lys Lys Ala Phe Glu Glu Ser Leu Ser Thr
    450                 455                 460

Leu Lys Trp Met Asp Glu Glu Thr Arg Lys Ser Ala Lys Glu Lys Ala
465                 470                 475                 480

Asp Ala Ile Tyr Asn Met Ile Gly Tyr Pro Asn Phe Ile Met Asp Pro
                485                 490                 495

Lys Glu Leu Asp Lys Val Phe Asn Asp Tyr Thr Ala Val Pro Asp Leu
            500                 505                 510

Tyr Phe Glu Asn Ala Met Arg Phe Phe Asn Phe Ser Trp Arg Val Thr
        515                 520                 525

Ala Asp Gln Leu Arg Lys Ala Pro Asn Arg Asp Gln Trp Ser Met Thr
    530                 535                 540

Pro Pro Met Val Asn Ala Tyr Tyr Ser Pro Thr Lys Asn Glu Ile Val
545                 550                 555                 560

Phe Pro Ala Gly Ile Leu Gln Ala Pro Phe Tyr Thr Arg Ser Ser Pro
                565                 570                 575

Lys Ala Leu Asn Phe Gly Gly Ile Gly Val Val Val Gly His Glu Leu
            580                 585                 590

Thr His Ala Phe Asp Asp Gln Gly Arg Glu Tyr Asp Lys Asp Gly Asn
        595                 600                 605

Leu Arg Pro Trp Trp Lys Asn Ser Ser Val Glu Ala Phe Lys Arg Gln
    610                 615                 620

Thr Glu Cys Met Val Glu Gln Tyr Ser Asn Tyr Ser Val Asn Gly Glu
625                 630                 635                 640

Pro Val Asn Gly Arg His Thr Leu Gly Glu Asn Ile Ala Asp Asn Gly
                645                 650                 655

Gly Leu Lys Ala Ala Tyr Arg Ala Tyr Gln Asn Trp Val Lys Lys Asn
            660                 665                 670

Gly Ala Glu His Ser Leu Pro Thr Leu Gly Leu Thr Asn Asn Gln Leu
        675                 680                 685

Phe Phe Leu Gly Phe Ala Gln Val Trp Cys Ser Val Arg Thr Pro Glu
    690                 695                 700

Ser Ser His Glu Gly Leu Ile Thr Asp Pro His Ser Pro Ser Arg Phe
705                 710                 715                 720

Arg Val Ile Gly Ser Leu Ser Asn Ser Lys Glu Phe Ser Glu His Phe
                725                 730                 735

Arg Cys Pro Pro Gly Ser Pro Met Asn Pro Pro His Lys Cys Glu Val
            740                 745                 750

Trp
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<210> SEQ ID NO 5
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (14)
<223> OTHER INFORMATION: i
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (18)
<223> OTHER INFORMATION: i
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (21)
<223> OTHER INFORMATION: i
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:
Oligonucleotide primers for RT-PCR reactions

<400> SEQUENCE: 5

tggatggatc gacngganac naca 24

<210> SEQ ID NO 6
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (14)
<223> OTHER INFORMATION: i
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (18)
<223> OTHER INFORMATION: i
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (21)
<223> OTHER INFORMATION: i
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:
Oligonucleotide primers for RT-PCR reactions

<400> SEQUENCE: 6

tggatggatc gacngganac nacg 24

<210> SEQ ID NO 7
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (4)
<223> OTHER INFORMATION: i
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (7)
<223> OTHER INFORMATION: i
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (14)
<223> OTHER INFORMATION: i
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (17)
<223> OTHER INFORMATION: i
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (20)
<223> OTHER INFORMATION: i
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (25)
<223> OTHER INFORMATION: i
<220> FEATURE:

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<223> OTHER INFORMATION: Description of Artificial Sequence:
Oligonucleotide primers for RT-PCR reactions

<400> SEQUENCE: 7

agtngtnttt cccngcnggn agtancttat ca 32

<210> SEQ ID NO 8
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (4)
<223> OTHER INFORMATION: i
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (7)
<223> OTHER INFORMATION: i
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (14)
<223> OTHER INFORMATION: i
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (17)
<223> OTHER INFORMATION: i
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (20)
<223> OTHER INFORMATION: i
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (25)
<223> OTHER INFORMATION: i
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:
Oligonucleotide primers for RT-PCR reactions

<400> SEQUENCE: 8

agtngtnttt cccngcnggn agtancttgc ca 32

<210> SEQ ID NO 9
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (2)
<223> OTHER INFORMATION: i
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (6)
<223> OTHER INFORMATION: i
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (9)
<223> OTHER INFORMATION: i
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (18)
<223> OTHER INFORMATION: i
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (21)
<223> OTHER INFORMATION: i
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:
Oligonucleotide primers for RT-PCR reactions
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (3)
<223> OTHER INFORMATION: i

<400> SEQUENCE: 9

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annccnccna tctagtcngc ngactagttt ctc 33

<210> SEQ ID NO 10
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (12)
<223> OTHER INFORMATION: i
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (24)
<223> OTHER INFORMATION: i
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:
Oligonucleotide primers for RT-PCR reactions

<400> SEQUENCE: 10

gatcaatctc tngatcgaag tctnaatctg gatgg 35

<210> SEQ ID NO 11
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (9)
<223> OTHER INFORMATION: i
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (12)
<223> OTHER INFORMATION: i
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (31)
<223> OTHER INFORMATION: i
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:
Oligonucleotide primers for RT-PCR reactions

<400> SEQUENCE: 11

tctcaccana tnctgagcat cgttcttcat ngggatg 37

<210> SEQ ID NO 12
<211> LENGTH: 2925
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (332)..(2626)

<400> SEQUENCE: 12

cgggagccag caccgtgtga cctcacaccc agctcagctg ctctactcca cccggagccc 60

accttggcca gctcacccca actctgagac atcccaacct agcctttaag gacttgccta 120

gcagtgactg agagcaccag ggtcccctgg gcacttgggg cacagcttac agcattgaga 180

gcagagacca ggacagtgca ccagcttcag tgtgtcctag gcatccgatc cgggctccag 240

ctgcctctct cctagccctg gcctgggggg cttagcggtg tgccttccac ccagaaccgg 300

ctgataggga aagtctgaga gcccagtggg g atg gtg gag aga gca ggc tgg 352
Met Val Glu Arg Ala Gly Trp
1 5

tgt cgg aag aag tcc cca ggc ttc gtg gag tat ggg ctg atg gtg ctg 400
Cys Arg Lys Lys Ser Pro Gly Phe Val Glu Tyr Gly Leu Met Val Leu
10 15 20

ctg ctg ctg ttg ctg gga gcc ata gtg act ctg ggt gtc ttc tac agc 448
Leu Leu Leu Leu Leu Gly Ala Ile Val Thr Leu Gly Val Phe Tyr Ser

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     25                  30                  35

ata ggg aag cag ctg ccc ctc tta act agc ctg cta cac ttc tcc tgg      496
Ile Gly Lys Gln Leu Pro Leu Leu Thr Ser Leu Leu His Phe Ser Trp
 40                  45                  50                  55

gat gag agg acg gtt gta aaa cga gcc ctc agg gat tca tca ctg aaa      544
Asp Glu Arg Thr Val Val Lys Arg Ala Leu Arg Asp Ser Ser Leu Lys
                 60                  65                  70

agt gac atc tgc acc acc cca agc tgt gtg ata gca gct gcc aga atc      592
Ser Asp Ile Cys Thr Thr Pro Ser Cys Val Ile Ala Ala Ala Arg Ile
             75                  80                  85

ctc gaa aac atg gac caa tcg agg aac ccc tgt gaa aac ttc tac cag      640
Leu Glu Asn Met Asp Gln Ser Arg Asn Pro Cys Glu Asn Phe Tyr Gln
         90                  95                 100

tac gcc tgc gga ggc tgg ctg agg cac cac gtg atc cca gag acc aac      688
Tyr Ala Cys Gly Gly Trp Leu Arg His His Val Ile Pro Glu Thr Asn
    105                 110                 115

tcc cga tac agc gtc ttt gac atc ctg cgg gac gag ctg gag gtt atc      736
Ser Arg Tyr Ser Val Phe Asp Ile Leu Arg Asp Glu Leu Glu Val Ile
120                 125                 130                 135

ctc aaa ggg gtg ctg gag gat tcc act tcc cag cat cgc ccg gcc gtg      784
Leu Lys Gly Val Leu Glu Asp Ser Thr Ser Gln His Arg Pro Ala Val
                140                 145                 150

gag aag gcc aag aca cta tat cgc tcc tgc atg aac caa agt gtg atc      832
Glu Lys Ala Lys Thr Leu Tyr Arg Ser Cys Met Asn Gln Ser Val Ile
            155                 160                 165

gag aag aga gac tct gag ccc ctg ctg agc gtc tta aaa atg gta gga      880
Glu Lys Arg Asp Ser Glu Pro Leu Leu Ser Val Leu Lys Met Val Gly
        170                 175                 180

ggt tgg cct gtg gcc atg gat aag tgg aac gag acc atg ggc ctc aag      928
Gly Trp Pro Val Ala Met Asp Lys Trp Asn Glu Thr Met Gly Leu Lys
    185                 190                 195

tgg gaa ctg gag cga cag ttg gct gtg ttg aac tcg cag ttc aac agg      976
Trp Glu Leu Glu Arg Gln Leu Ala Val Leu Asn Ser Gln Phe Asn Arg
200                 205                 210                 215

cgg gtc ctc atc gac ctc ttc atc tgg aat gac gac cag aac tcc agc     1024
Arg Val Leu Ile Asp Leu Phe Ile Trp Asn Asp Asp Gln Asn Ser Ser
                220                 225                 230

cgg cat gtc atc tac ata gac cag ccc acc ttg ggc atg cca tcc cgg     1072
Arg His Val Ile Tyr Ile Asp Gln Pro Thr Leu Gly Met Pro Ser Arg
            235                 240                 245

gag tac tat ttc cag gag gac aac aac cac aag gta cgg aaa gcc tac     1120
Glu Tyr Tyr Phe Gln Glu Asp Asn Asn His Lys Val Arg Lys Ala Tyr
        250                 255                 260

ctg gag ttc atg acg tca gtg gcc act atg ctt agg aaa gac cag aac     1168
Leu Glu Phe Met Thr Ser Val Ala Thr Met Leu Arg Lys Asp Gln Asn
    265                 270                 275

ctg tcc aag gag agc gcc atg gtg cgg gag gag atg gcg gag gtg ctg     1216
Leu Ser Lys Glu Ser Ala Met Val Arg Glu Glu Met Ala Glu Val Leu
280                 285                 290                 295

gaa ctg gag acg cat ctg gcc aac gcc aca gtc ccc cag gag aaa agg     1264
Glu Leu Glu Thr His Leu Ala Asn Ala Thr Val Pro Gln Glu Lys Arg
                300                 305                 310

cat gat gtc act gcc ctg tac cac cga atg gac ctg atg gag cta cag     1312
His Asp Val Thr Ala Leu Tyr His Arg Met Asp Leu Met Glu Leu Gln
            315                 320                 325

gaa agg ttt ggt ctg aag ggg ttt aac tgg act ctc ttc ata caa aac     1360
Glu Arg Phe Gly Leu Lys Gly Phe Asn Trp Thr Leu Phe Ile Gln Asn
        330                 335                 340

gtg ttg tct tct gtg gaa gtc gag ctg ttc cca gat gag gag gtg gtg     1408
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Val Leu Ser Ser Val Glu Val Glu Leu Phe Pro Asp Glu Glu Val Val
    345                 350                 355

gtc tac ggc atc ccc tac ctg gag aat ctg gag gat atc att gat agc     1456
Val Tyr Gly Ile Pro Tyr Leu Glu Asn Leu Glu Asp Ile Ile Asp Ser
360                 365                 370                 375

tac tca gca cgg acc atg cag aac tac ctg gta tgg cgc ctg gtg cta     1504
Tyr Ser Ala Arg Thr Met Gln Asn Tyr Leu Val Trp Arg Leu Val Leu
                380                 385                 390

gat cga att ggc agc ctg agc cag aga ttc aaa gag gcg cgt gtg gac     1552
Asp Arg Ile Gly Ser Leu Ser Gln Arg Phe Lys Glu Ala Arg Val Asp
            395                 400                 405

tac cgc aag gcg ctg tac ggc acg acc gtg gag gag gta cgc tgg cga     1600
Tyr Arg Lys Ala Leu Tyr Gly Thr Thr Val Glu Glu Val Arg Trp Arg
        410                 415                 420

gag tgt gtc agc tat gtc aac agt aac atg gag agc gcc gtg ggc tcc     1648
Glu Cys Val Ser Tyr Val Asn Ser Asn Met Glu Ser Ala Val Gly Ser
    425                 430                 435

ctc tac atc aag cgg gcc ttc tcc aag gac agc aag agc acg gtc aga     1696
Leu Tyr Ile Lys Arg Ala Phe Ser Lys Asp Ser Lys Ser Thr Val Arg
440                 445                 450                 455

gag ctg att gag aag ata agg tcc gtg ttt gtg gat aac ctg gat gag     1744
Glu Leu Ile Glu Lys Ile Arg Ser Val Phe Val Asp Asn Leu Asp Glu
                460                 465                 470

ctg aac tgg atg gac gag gaa tcc aag aag aag gcc cag gaa aag gcc     1792
Leu Asn Trp Met Asp Glu Glu Ser Lys Lys Lys Ala Gln Glu Lys Ala
            475                 480                 485

atg aat ata cgg gaa cag att ggc tac cct gac tac att ttg gaa gat     1840
Met Asn Ile Arg Glu Gln Ile Gly Tyr Pro Asp Tyr Ile Leu Glu Asp
        490                 495                 500

aac aat aaa cac ctg gat gag gaa tac tcc agt ttg act ttc tat gag     1888
Asn Asn Lys His Leu Asp Glu Glu Tyr Ser Ser Leu Thr Phe Tyr Glu
    505                 510                 515

gac ctg tat ttt gag aac gga ctt cag aac ctc aag aac aat gcc cag     1936
Asp Leu Tyr Phe Glu Asn Gly Leu Gln Asn Leu Lys Asn Asn Ala Gln
520                 525                 530                 535

agg agc ctc aag aag ctt cgg gaa aag gtg gac cag aat ctc tgg atc     1984
Arg Ser Leu Lys Lys Leu Arg Glu Lys Val Asp Gln Asn Leu Trp Ile
                540                 545                 550

atc ggg gct gca gtg gtc aat gca ttc tac tcc cca aac aga aac cag     2032
Ile Gly Ala Ala Val Val Asn Ala Phe Tyr Ser Pro Asn Arg Asn Gln
            555                 560                 565

atc gtc ttt cca gca ggg att ctc cag ccg ccc ttc ttc agc aag gac     2080
Ile Val Phe Pro Ala Gly Ile Leu Gln Pro Pro Phe Phe Ser Lys Asp
        570                 575                 580

caa cca cag tcc ttg aat ttt ggg ggc atc ggg atg gtg att ggg cac     2128
Gln Pro Gln Ser Leu Asn Phe Gly Gly Ile Gly Met Val Ile Gly His
    585                 590                 595

gag atc aca cac ggc ttt gat gat aat ggt cgt aac ttt gac aag aac     2176
Glu Ile Thr His Gly Phe Asp Asp Asn Gly Arg Asn Phe Asp Lys Asn
600                 605                 610                 615

ggc aac atg ctg gac tgg tgg agt aac ttc tcg gcc cgg cac ttc caa     2224
Gly Asn Met Leu Asp Trp Trp Ser Asn Phe Ser Ala Arg His Phe Gln
                620                 625                 630

cag cag tcg caa tgc atg atc tat cag tac ggc aac ttc tct tgg gaa     2272
Gln Gln Ser Gln Cys Met Ile Tyr Gln Tyr Gly Asn Phe Ser Trp Glu
            635                 640                 645

cta gca gac aac cag aat gtg aac gga ttc agt acc ctc ggg gag aac     2320
Leu Ala Asp Asn Gln Asn Val Asn Gly Phe Ser Thr Leu Gly Glu Asn
        650                 655                 660
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att gcc gac aac gga ggt gtg cga cag gca tac aag gct tac cta cgg     2368
Ile Ala Asp Asn Gly Gly Val Arg Gln Ala Tyr Lys Ala Tyr Leu Arg
    665                 670                 675

tgg ctg gct gat ggc ggc aaa gat cag cga ctg ccg gga ctg aac ctg     2416
Trp Leu Ala Asp Gly Gly Lys Asp Gln Arg Leu Pro Gly Leu Asn Leu
680                 685                 690                 695

acc tat gcc cag ctt ttc ttc atc aac tat gcc cag gtg tgg tgt ggg     2464
Thr Tyr Ala Gln Leu Phe Phe Ile Asn Tyr Ala Gln Val Trp Cys Gly
                700                 705                 710

tcc tat agg ccg gag ttc gcc gtc cag tcc atc aag acg gac gtc cac     2512
Ser Tyr Arg Pro Glu Phe Ala Val Gln Ser Ile Lys Thr Asp Val His
            715                 720                 725

agt cct ctt aag tac agg gtg ctg ggc tca cta cag aac ctg cca ggc     2560
Ser Pro Leu Lys Tyr Arg Val Leu Gly Ser Leu Gln Asn Leu Pro Gly
        730                 735                 740

ttc tct gag gca ttc cac tgc cca cga ggc agc ccc atg cac ccc atg     2608
Phe Ser Glu Ala Phe His Cys Pro Arg Gly Ser Pro Met His Pro Met
    745                 750                 755

aag cga tgt cgc atc tgg tagccaaggc tgagctatgc tgcggcccac            2656
Lys Arg Cys Arg Ile Trp
760                 765

gccccgccac ccagaggctt cgcgaatggt gtagctggca gagatgtgca ggtctttgcc   2716

tgaaggccac cggagccacc agccagccct ccgcgcccag cctagagtgt agccacccgc   2776

ccacacccgg gatgagtggt gccggtcctg cgccccctca ggccagtgag ggtcagcagc   2836

ccaggaagag cagtcagctg ccttccaccc tctccatagt gtgtggctaa atgttctcga   2896

gcttcagact tgagctaagt aaacgcttc                                     2925


<210> SEQ ID NO 13
<211> LENGTH: 765
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 13

Met Val Glu Arg Ala Gly Trp Cys Arg Lys Lys Ser Pro Gly Phe Val
  1               5                  10                  15

Glu Tyr Gly Leu Met Val Leu Leu Leu Leu Leu Gly Ala Ile Val
             20                  25                  30

Thr Leu Gly Val Phe Tyr Ser Ile Gly Lys Gln Leu Pro Leu Leu Thr
         35                  40                  45

Ser Leu Leu His Phe Ser Trp Asp Glu Arg Thr Val Val Lys Arg Ala
     50                  55                  60

Leu Arg Asp Ser Ser Leu Lys Ser Asp Ile Cys Thr Thr Pro Ser Cys
 65                  70                  75                  80

Val Ile Ala Ala Ala Arg Ile Leu Glu Asn Met Asp Gln Ser Arg Asn
                 85                  90                  95

Pro Cys Glu Asn Phe Tyr Gln Tyr Ala Cys Gly Gly Trp Leu Arg His
            100                 105                 110

His Val Ile Pro Glu Thr Asn Ser Arg Tyr Ser Val Phe Asp Ile Leu
        115                 120                 125

Arg Asp Glu Leu Glu Val Ile Leu Lys Gly Val Leu Glu Asp Ser Thr
    130                 135                 140

Ser Gln His Arg Pro Ala Val Glu Lys Ala Lys Thr Leu Tyr Arg Ser
145                 150                 155                 160

Cys Met Asn Gln Ser Val Ile Glu Lys Arg Asp Ser Glu Pro Leu Leu
                165                 170                 175
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Ser Val Leu Lys Met Val Gly Gly Trp Pro Val Ala Met Asp Lys Trp
            180                 185                 190

Asn Glu Thr Met Gly Leu Lys Trp Glu Leu Glu Arg Gln Leu Ala Val
        195                 200                 205

Leu Asn Ser Gln Phe Asn Arg Arg Val Leu Ile Asp Leu Phe Ile Trp
    210                 215                 220

Asn Asp Asp Gln Asn Ser Ser Arg His Val Ile Tyr Ile Asp Gln Pro
225                 230                 235                 240

Thr Leu Gly Met Pro Ser Arg Glu Tyr Tyr Phe Gln Glu Asp Asn Asn
                245                 250                 255

His Lys Val Arg Lys Ala Tyr Leu Glu Phe Met Thr Ser Val Ala Thr
            260                 265                 270

Met Leu Arg Lys Asp Gln Asn Leu Ser Lys Glu Ser Ala Met Val Arg
        275                 280                 285

Glu Glu Met Ala Glu Val Leu Glu Leu Glu Thr His Leu Ala Asn Ala
    290                 295                 300

Thr Val Pro Gln Glu Lys Arg His Asp Val Thr Ala Leu Tyr His Arg
305                 310                 315                 320

Met Asp Leu Met Glu Leu Gln Glu Arg Phe Gly Leu Lys Gly Phe Asn
                325                 330                 335

Trp Thr Leu Phe Ile Gln Asn Val Leu Ser Ser Val Glu Val Glu Leu
            340                 345                 350

Phe Pro Asp Glu Glu Val Val Val Tyr Gly Ile Pro Tyr Leu Glu Asn
        355                 360                 365

Leu Glu Asp Ile Ile Asp Ser Tyr Ser Ala Arg Thr Met Gln Asn Tyr
    370                 375                 380

Leu Val Trp Arg Leu Val Leu Asp Arg Ile Gly Ser Leu Ser Gln Arg
385                 390                 395                 400

Phe Lys Glu Ala Arg Val Asp Tyr Arg Lys Ala Leu Tyr Gly Thr Thr
                405                 410                 415

Val Glu Glu Val Arg Trp Arg Glu Cys Val Ser Tyr Val Asn Ser Asn
            420                 425                 430

Met Glu Ser Ala Val Gly Ser Leu Tyr Ile Lys Arg Ala Phe Ser Lys
        435                 440                 445

Asp Ser Lys Ser Thr Val Arg Glu Leu Ile Glu Lys Ile Arg Ser Val
    450                 455                 460

Phe Val Asp Asn Leu Asp Glu Leu Asn Trp Met Asp Glu Glu Ser Lys
465                 470                 475                 480

Lys Lys Ala Gln Glu Lys Ala Met Asn Ile Arg Glu Gln Ile Gly Tyr
                485                 490                 495

Pro Asp Tyr Ile Leu Glu Asp Asn Asn Lys His Leu Asp Glu Glu Tyr
            500                 505                 510

Ser Ser Leu Thr Phe Tyr Glu Asp Leu Tyr Phe Glu Asn Gly Leu Gln
        515                 520                 525

Asn Leu Lys Asn Asn Ala Gln Arg Ser Leu Lys Lys Leu Arg Glu Lys
    530                 535                 540

Val Asp Gln Asn Leu Trp Ile Ile Gly Ala Ala Val Val Asn Ala Phe
545                 550                 555                 560

Tyr Ser Pro Asn Arg Asn Gln Ile Val Phe Pro Ala Gly Ile Leu Gln
                565                 570                 575

Pro Pro Phe Phe Ser Lys Asp Gln Pro Gln Ser Leu Asn Phe Gly Gly
            580                 585                 590

Ile Gly Met Val Ile Gly His Glu Ile Thr His Gly Phe Asp Asp Asn
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595 600 605

Gly Arg Asn Phe Asp Lys Asn Gly Asn Met Leu Asp Trp Trp Ser Asn
610 615 620

Phe Ser Ala Arg His Phe Gln Gln Gln Ser Gln Cys Met Ile Tyr Gln
625 630 635 640

Tyr Gly Asn Phe Ser Trp Glu Leu Ala Asp Asn Gln Asn Val Asn Gly
645 650 655

Phe Ser Thr Leu Gly Glu Asn Ile Ala Asp Asn Gly Gly Val Arg Gln
660 665 670

Ala Tyr Lys Ala Tyr Leu Arg Trp Leu Ala Asp Gly Gly Lys Asp Gln
675 680 685

Arg Leu Pro Gly Leu Asn Leu Thr Tyr Ala Gln Leu Phe Phe Ile Asn
690 695 700

Tyr Ala Gln Val Trp Cys Gly Ser Tyr Arg Pro Glu Phe Ala Val Gln
705 710 715 720

Ser Ile Lys Thr Asp Val His Ser Pro Leu Lys Tyr Arg Val Leu Gly
725 730 735

Ser Leu Gln Asn Leu Pro Gly Phe Ser Glu Ala Phe His Cys Pro Arg
740 745 750

Gly Ser Pro Met His Pro Met Lys Arg Cys Arg Ile Trp
755 760 765

<210> SEQ ID NO 14
<211> LENGTH: 2676
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (7)..(2316)

<400> SEQUENCE: 14

gtgggg atg gtg gag agc gcc ggc cgt gca ggg cag aag cgc ccg ggg 48
Met Val Glu Ser Ala Gly Arg Ala Gly Gln Lys Arg Pro Gly
1 5 10

ttc ctg gag ggg ggg ctg ctg ctg ctg ctg ctg gtg acc gct gcc 96
Phe Leu Glu Gly Gly Leu Leu Leu Leu Leu Leu Val Thr Ala Ala
15 20 25 30

ctg gtg gcc ttg ggt gtc ctc tac gcc gac cgc aga ggg aag cag ctg 144
Leu Val Ala Leu Gly Val Leu Tyr Ala Asp Arg Arg Gly Lys Gln Leu
35 40 45

cca cgc ctt gct agc cgg ctg tgc ttc tta cag gag gag agg acc ttt 192
Pro Arg Leu Ala Ser Arg Leu Cys Phe Leu Gln Glu Glu Arg Thr Phe
50 55 60

gta aaa cga aaa ccc cga ggg atc cca gag gcc caa gag gtg agc gag 240
Val Lys Arg Lys Pro Arg Gly Ile Pro Glu Ala Gln Glu Val Ser Glu
65 70 75

gtc tgc acc acc cct ggc tgc gtg ata gca gcc gcc agg atc ctc cag 288
Val Cys Thr Thr Pro Gly Cys Val Ile Ala Ala Ala Arg Ile Leu Gln
80 85 90

aac atg gac ccg acc acg gaa ccg tgt gac gac ttc tac cag ttt gca 336
Asn Met Asp Pro Thr Thr Glu Pro Cys Asp Asp Phe Tyr Gln Phe Ala
95 100 105 110

tgc gga ggc tgg ctg cgg cgc cac gtg atc cct gag acc aac tca aga 384
Cys Gly Gly Trp Leu Arg Arg His Val Ile Pro Glu Thr Asn Ser Arg
115 120 125

tac agc atc ttt gac gtc ctc cgc gac gag ctg gag gtc atc ctc aaa 432
Tyr Ser Ile Phe Asp Val Leu Arg Asp Glu Leu Glu Val Ile Leu Lys
130 135 140

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gcg gtg ctg gag aat tcg act gcc aag gac cgg ccg gct gtg gag aag      480
Ala Val Leu Glu Asn Ser Thr Ala Lys Asp Arg Pro Ala Val Glu Lys
        145                 150                 155

gcc agg acg ctg tac cgc tcc tgc atg aac cag agt gtg ata gag aag      528
Ala Arg Thr Leu Tyr Arg Ser Cys Met Asn Gln Ser Val Ile Glu Lys
    160                 165                 170

cga ggc tct cag ccc ctg ctg gac atc ttg gag gtg gtg gga ggc tgg      576
Arg Gly Ser Gln Pro Leu Leu Asp Ile Leu Glu Val Val Gly Gly Trp
175                 180                 185                 190

ccg gtg gcg atg gac agg tgg aac gag acc gta gga ctc gag tgg gag      624
Pro Val Ala Met Asp Arg Trp Asn Glu Thr Val Gly Leu Glu Trp Glu
                195                 200                 205

ctg gag cgg cag ctg gcg ctg atg aac tca cag ttc aac agg cgc gtc      672
Leu Glu Arg Gln Leu Ala Leu Met Asn Ser Gln Phe Asn Arg Arg Val
            210                 215                 220

ctc atc gac ctc ttc atc tgg aac gac gac cag aac tcc agc cgg cac      720
Leu Ile Asp Leu Phe Ile Trp Asn Asp Asp Gln Asn Ser Ser Arg His
        225                 230                 235

atc atc tac ata gac cag ccc acc ttg ggc atg ccc tcc cga gag tac      768
Ile Ile Tyr Ile Asp Gln Pro Thr Leu Gly Met Pro Ser Arg Glu Tyr
    240                 245                 250

tac ttc aac ggc ggc agc aac cgg aag gtg cgg gaa gcc tac ctg cag      816
Tyr Phe Asn Gly Gly Ser Asn Arg Lys Val Arg Glu Ala Tyr Leu Gln
255                 260                 265                 270

ttc atg gtg tca gtg gcc acg ttg ctg cgg gag gat gca aac ctg ccc      864
Phe Met Val Ser Val Ala Thr Leu Leu Arg Glu Asp Ala Asn Leu Pro
                275                 280                 285

agg gac agc tgc ctg gtg cag gag gac atg gtg cag gtt ctg gag ctg      912
Arg Asp Ser Cys Leu Val Gln Glu Asp Met Val Gln Val Leu Glu Leu
            290                 295                 300

gag aca cag ctg gcc aag gcc acg gta ccc cag gag gag aga cac gac      960
Glu Thr Gln Leu Ala Lys Ala Thr Val Pro Gln Glu Glu Arg His Asp
        305                 310                 315

gtc atc gcc ttg tac cac cgg atg gga ctg gag gag ctg caa agc cag     1008
Val Ile Ala Leu Tyr His Arg Met Gly Leu Glu Glu Leu Gln Ser Gln
    320                 325                 330

ttt ggc ctg aag gga ttt aac tgg act ctg ttc ata caa act gtg cta     1056
Phe Gly Leu Lys Gly Phe Asn Trp Thr Leu Phe Ile Gln Thr Val Leu
335                 340                 345                 350

tcc tct gtc aaa atc aag ctg ctg cca gat gag gaa gtg gtg gtc tat     1104
Ser Ser Val Lys Ile Lys Leu Leu Pro Asp Glu Glu Val Val Val Tyr
                355                 360                 365

ggc atc ccc tac ctg cag aac ctt gaa aac atc atc gac acc tac tca     1152
Gly Ile Pro Tyr Leu Gln Asn Leu Glu Asn Ile Ile Asp Thr Tyr Ser
            370                 375                 380

gcc agg acc ata cag aac tac ctg gtc tgg cgc ctg gtg ctg gac cgc     1200
Ala Arg Thr Ile Gln Asn Tyr Leu Val Trp Arg Leu Val Leu Asp Arg
        385                 390                 395

att ggt agc cta agc cag aga ttc aag gac aca cga gtg aac tac cgc     1248
Ile Gly Ser Leu Ser Gln Arg Phe Lys Asp Thr Arg Val Asn Tyr Arg
    400                 405                 410

aag gcg ctg ttt ggc aca atg gtg gag gag gtg cgc tgg cgt gaa tgt     1296
Lys Ala Leu Phe Gly Thr Met Val Glu Glu Val Arg Trp Arg Glu Cys
415                 420                 425                 430

gtg ggc tac gtc aac agc aac atg gag aac gcc gtg ggc tcc ctc tac     1344
Val Gly Tyr Val Asn Ser Asn Met Glu Asn Ala Val Gly Ser Leu Tyr
                435                 440                 445

gtc agg gag gcg ttc cct gga gac agc aag agc atg gtc aga gaa ctc     1392
Val Arg Glu Ala Phe Pro Gly Asp Ser Lys Ser Met Val Arg Glu Leu
            450                 455                 460
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att gac aag gtg cgg aca gtg ttt gtg gag acg ctg gac gag ctg ggc     1440
Ile Asp Lys Val Arg Thr Val Phe Val Glu Thr Leu Asp Glu Leu Gly
        465                 470                 475

tgg atg gac gag gag tcc aag aag aag gcg cag gag aag gcc atg agc     1488
Trp Met Asp Glu Glu Ser Lys Lys Lys Ala Gln Glu Lys Ala Met Ser
    480                 485                 490

atc cgg gag cag atc ggg cac cct gac tac atc ctg gag gag atg aac     1536
Ile Arg Glu Gln Ile Gly His Pro Asp Tyr Ile Leu Glu Glu Met Asn
495                 500                 505                 510

agg cgc ctg gac gag gag tac tcc aat ctg aac ttc tca gag gac ctg     1584
Arg Arg Leu Asp Glu Glu Tyr Ser Asn Leu Asn Phe Ser Glu Asp Leu
                515                 520                 525

tac ttt gag aac agt ctg cag aac ctc aag gtg ggc gcc cag cgg agc     1632
Tyr Phe Glu Asn Ser Leu Gln Asn Leu Lys Val Gly Ala Gln Arg Ser
            530                 535                 540

ctc agg aag ctt cgg gaa aag gtg gac cca aat ctc tgg atc atc ggg     1680
Leu Arg Lys Leu Arg Glu Lys Val Asp Pro Asn Leu Trp Ile Ile Gly
        545                 550                 555

gcg gcg gtg gtc aat gcg ttc tac tcc cca aac cga aac cag att gta     1728
Ala Ala Val Val Asn Ala Phe Tyr Ser Pro Asn Arg Asn Gln Ile Val
    560                 565                 570

ttc cct gcc ggg atc ctc cag ccc ccc ttc ttc agc aag gag cag cca     1776
Phe Pro Ala Gly Ile Leu Gln Pro Pro Phe Phe Ser Lys Glu Gln Pro
575                 580                 585                 590

cag gcc ttg aac ttt gga ggc att ggg atg gtg atc ggg cac gag atc     1824
Gln Ala Leu Asn Phe Gly Gly Ile Gly Met Val Ile Gly His Glu Ile
                595                 600                 605

acg cac ggc ttt gac gac aat ggc cgg aac ttc gac aag aat ggc aac     1872
Thr His Gly Phe Asp Asp Asn Gly Arg Asn Phe Asp Lys Asn Gly Asn
            610                 615                 620

atg atg gat tgg tgg agt aac ttc tcc acc cag cac ttc cgg gag cag     1920
Met Met Asp Trp Trp Ser Asn Phe Ser Thr Gln His Phe Arg Glu Gln
        625                 630                 635

tca gag tgc atg atc tac cag tac ggc aac tac tcc tgg gac ctg gca     1968
Ser Glu Cys Met Ile Tyr Gln Tyr Gly Asn Tyr Ser Trp Asp Leu Ala
    640                 645                 650

gac gaa cag aac gtg aac gga ttc aac acc ctt ggg gaa aac att gct     2016
Asp Glu Gln Asn Val Asn Gly Phe Asn Thr Leu Gly Glu Asn Ile Ala
655                 660                 665                 670

gac aac gga ggg gtg cgg caa gcc tat aag gcc tac ctc aag tgg atg     2064
Asp Asn Gly Gly Val Arg Gln Ala Tyr Lys Ala Tyr Leu Lys Trp Met
                675                 680                 685

gca gag ggt ggc aag gac cag cag ctg ccc ggc ctg gat ctc acc cat     2112
Ala Glu Gly Gly Lys Asp Gln Gln Leu Pro Gly Leu Asp Leu Thr His
            690                 695                 700

gag cag ctc ttc ttc atc aac tat gcc cag gtg tgg tgc ggg tcc tac     2160
Glu Gln Leu Phe Phe Ile Asn Tyr Ala Gln Val Trp Cys Gly Ser Tyr
        705                 710                 715

cgg ccc gag ttc gcc atc caa tcc atc aag aca gac gtc cac agt ccc     2208
Arg Pro Glu Phe Ala Ile Gln Ser Ile Lys Thr Asp Val His Ser Pro
    720                 725                 730

ctg aag tac agg gta ctg ggg tcg ctg cag aac ctg gcc gcc ttc gca     2256
Leu Lys Tyr Arg Val Leu Gly Ser Leu Gln Asn Leu Ala Ala Phe Ala
735                 740                 745                 750

gac acg ttc cac tgt gcc cgg ggc acc ccc atg cac ccc aag gag cga     2304
Asp Thr Phe His Cys Ala Arg Gly Thr Pro Met His Pro Lys Glu Arg
                755                 760                 765

tgc cgc gtg tgg tagccaaggc cctgccgcgc tgtgcggccc acgcccaccc         2356
Cys Arg Val Trp
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770

gctgctcgga ggcatctgtg cgaaggtgca gctagcggcg acccagtgta cgtcccgccc 2416

cggccaacca tgccaagcct gcctgccagg cctctgcgcc tggcctaggg tgcagccacc 2476

tgcctgacac ccagggatga gcagtgtcca gtgcagtacc tggaccggag ccccccttcac 2536

agacacccgc ggggctcagt gcccccgtca caactctgta gagacaatca actgtgtcct 2596

gcccaccctt caaggtgcat tgtcttccag tatctacagc ttcagaactt gagctaagta 2656

aatgctttca aagaaaaaaa 2676

<210> SEQ ID NO 15
<211> LENGTH: 770
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 15

Met Val Glu Ser Ala Gly Arg Ala Gly Gln Lys Arg Pro Gly Phe Leu
1 5 10 15

Glu Gly Gly Leu Leu Leu Leu Leu Leu Leu Val Thr Ala Ala Leu Val
20 25 30

Ala Leu Gly Val Leu Tyr Ala Asp Arg Arg Gly Lys Gln Leu Pro Arg
35 40 45

Leu Ala Ser Arg Leu Cys Phe Leu Gln Glu Glu Arg Thr Phe Val Lys
50 55 60

Arg Lys Pro Arg Gly Ile Pro Glu Ala Gln Glu Val Ser Glu Val Cys
65 70 75 80

Thr Thr Pro Gly Cys Val Ile Ala Ala Ala Arg Ile Leu Gln Asn Met
85 90 95

Asp Pro Thr Thr Glu Pro Cys Asp Asp Phe Tyr Gln Phe Ala Cys Gly
100 105 110

Gly Trp Leu Arg Arg His Val Ile Pro Glu Thr Asn Ser Arg Tyr Ser
115 120 125

Ile Phe Asp Val Leu Arg Asp Glu Leu Glu Val Ile Leu Lys Ala Val
130 135 140

Leu Glu Asn Ser Thr Ala Lys Asp Arg Pro Ala Val Glu Lys Ala Arg
145 150 155 160

Thr Leu Tyr Arg Ser Cys Met Asn Gln Ser Val Ile Glu Lys Arg Gly
165 170 175

Ser Gln Pro Leu Leu Asp Ile Leu Glu Val Val Gly Gly Trp Pro Val
180 185 190

Ala Met Asp Arg Trp Asn Glu Thr Val Gly Leu Glu Trp Glu Leu Glu
195 200 205

Arg Gln Leu Ala Leu Met Asn Ser Gln Phe Asn Arg Arg Val Leu Ile
210 215 220

Asp Leu Phe Ile Trp Asn Asp Asp Gln Asn Ser Ser Arg His Ile Ile
225 230 235 240

Tyr Ile Asp Gln Pro Thr Leu Gly Met Pro Ser Arg Glu Tyr Tyr Phe
245 250 255

Asn Gly Gly Ser Asn Arg Lys Val Arg Glu Ala Tyr Leu Gln Phe Met
260 265 270

Val Ser Val Ala Thr Leu Leu Arg Glu Asp Ala Asn Leu Pro Arg Asp
275 280 285

Ser Cys Leu Val Gln Glu Asp Met Val Gln Val Leu Glu Leu Glu Thr
290 295 300

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Gln Leu Ala Lys Ala Thr Val Pro Gln Glu Glu Arg His Asp Val Ile
305                 310                 315                 320

Ala Leu Tyr His Arg Met Gly Leu Glu Glu Leu Gln Ser Gln Phe Gly
                325                 330                 335

Leu Lys Gly Phe Asn Trp Thr Leu Phe Ile Gln Thr Val Leu Ser Ser
            340                 345                 350

Val Lys Ile Lys Leu Leu Pro Asp Glu Glu Val Val Val Tyr Gly Ile
        355                 360                 365

Pro Tyr Leu Gln Asn Leu Glu Asn Ile Ile Asp Thr Tyr Ser Ala Arg
    370                 375                 380

Thr Ile Gln Asn Tyr Leu Val Trp Arg Leu Val Leu Asp Arg Ile Gly
385                 390                 395                 400

Ser Leu Ser Gln Arg Phe Lys Asp Thr Arg Val Asn Tyr Arg Lys Ala
                405                 410                 415

Leu Phe Gly Thr Met Val Glu Glu Val Arg Trp Arg Glu Cys Val Gly
            420                 425                 430

Tyr Val Asn Ser Asn Met Glu Asn Ala Val Gly Ser Leu Tyr Val Arg
        435                 440                 445

Glu Ala Phe Pro Gly Asp Ser Lys Ser Met Val Arg Glu Leu Ile Asp
    450                 455                 460

Lys Val Arg Thr Val Phe Val Glu Thr Leu Asp Glu Leu Gly Trp Met
465                 470                 475                 480

Asp Glu Glu Ser Lys Lys Lys Ala Gln Glu Lys Ala Met Ser Ile Arg
                485                 490                 495

Glu Gln Ile Gly His Pro Asp Tyr Ile Leu Glu Glu Met Asn Arg Arg
            500                 505                 510

Leu Asp Glu Glu Tyr Ser Asn Leu Asn Phe Ser Glu Asp Leu Tyr Phe
        515                 520                 525

Glu Asn Ser Leu Gln Asn Leu Lys Val Gly Ala Gln Arg Ser Leu Arg
    530                 535                 540

Lys Leu Arg Glu Lys Val Asp Pro Asn Leu Trp Ile Ile Gly Ala Ala
545                 550                 555                 560

Val Val Asn Ala Phe Tyr Ser Pro Asn Arg Asn Gln Ile Val Phe Pro
                565                 570                 575

Ala Gly Ile Leu Gln Pro Pro Phe Phe Ser Lys Glu Gln Pro Gln Ala
            580                 585                 590

Leu Asn Phe Gly Gly Ile Gly Met Val Ile Gly His Glu Ile Thr His
        595                 600                 605

Gly Phe Asp Asp Asn Gly Arg Asn Phe Asp Lys Asn Gly Asn Met Met
    610                 615                 620

Asp Trp Trp Ser Asn Phe Ser Thr Gln His Phe Arg Glu Gln Ser Glu
625                 630                 635                 640

Cys Met Ile Tyr Gln Tyr Gly Asn Tyr Ser Trp Asp Leu Ala Asp Glu
                645                 650                 655

Gln Asn Val Asn Gly Phe Asn Thr Leu Gly Glu Asn Ile Ala Asp Asn
            660                 665                 670

Gly Gly Val Arg Gln Ala Tyr Lys Ala Tyr Leu Lys Trp Met Ala Glu
        675                 680                 685

Gly Gly Lys Asp Gln Gln Leu Pro Gly Leu Asp Leu Thr His Glu Gln
    690                 695                 700

Leu Phe Phe Ile Asn Tyr Ala Gln Val Trp Cys Gly Ser Tyr Arg Pro
705                 710                 715                 720

Glu Phe Ala Ile Gln Ser Ile Lys Thr Asp Val His Ser Pro Leu Lys
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                725                 730                 735

Tyr Arg Val Leu Gly Ser Leu Gln Asn Leu Ala Ala Phe Ala Asp Thr
            740                 745                 750

Phe His Cys Ala Arg Gly Thr Pro Met His Pro Lys Glu Arg Cys Arg
        755                 760                 765

Val Trp
    770


<210> SEQ ID NO 16
<211> LENGTH: 2871
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (205)..(2529)

<400> SEQUENCE: 16

ggcgctggga gacaccggac gcccgctcgg ctgcgctgcg gctcaggccc ccgctcggcc     60

cgacccgctc ggtcaccgcc ggctcgggcg cgcacctgcc ggctgcggcc ccagggccat    120

gcggaggccc acgaggaggc cggcggccac gcgcatcccg tagcccaggt ggcccaggtc    180

tgcaccgcgg cggcctcggc gccg atg gag ccc ccg tat tcg ctg acg gcg       231
                          Met Glu Pro Pro Tyr Ser Leu Thr Ala
                            1               5

cac tac gat gag ttc caa gag gtc aag tac gtg agc cgc tgc ggc gcg      279
His Tyr Asp Glu Phe Gln Glu Val Lys Tyr Val Ser Arg Cys Gly Ala
 10                  15                  20                  25

ggg ggc gcg cgc ggg gcc tcc ctg ccc ccg ggc ttc ccg ttg ggc gct      327
Gly Gly Ala Arg Gly Ala Ser Leu Pro Pro Gly Phe Pro Leu Gly Ala
                 30                  35                  40

gcg cgc agc gcc acc ggg gcc cgg tcc ggg ctg ccg cgc tgg aac cgg      375
Ala Arg Ser Ala Thr Gly Ala Arg Ser Gly Leu Pro Arg Trp Asn Arg
             45                  50                  55

cgc gag gtg tgc ctg ctg tcg ggg ctg gtg ttc gcc gcc ggc ctc tgc      423
Arg Glu Val Cys Leu Leu Ser Gly Leu Val Phe Ala Ala Gly Leu Cys
         60                  65                  70

gcc att ctg gcg gct atg ctg gcc ctc aag tac ctg ggc ccg gtc gcg      471
Ala Ile Leu Ala Ala Met Leu Ala Leu Lys Tyr Leu Gly Pro Val Ala
     75                  80                  85

gcc ggc ggc ggc gcc tgt ccc gag ggc tgc cct gag cgc aag gcc ttc      519
Ala Gly Gly Gly Ala Cys Pro Glu Gly Cys Pro Glu Arg Lys Ala Phe
 90                  95                  100                 105

gcg cgc gcc gct cgc ttc ctg gcc gcc aac ctg gac gcc agc atc gac      567
Ala Arg Ala Ala Arg Phe Leu Ala Ala Asn Leu Asp Ala Ser Ile Asp
                110                 115                 120

cca tgc cag gac ttc tac tcg ttc gcc tgc ggc ggt tgg ctg cgg cgc      615
Pro Cys Gln Asp Phe Tyr Ser Phe Ala Cys Gly Gly Trp Leu Arg Arg
            125                 130                 135

cac gcc atc ccc gac gac aag ctc acc tat ggc acc atc gcg gca atc      663
His Ala Ile Pro Asp Asp Lys Leu Thr Tyr Gly Thr Ile Ala Ala Ile
        140                 145                 150

ggc gag caa aac gag gag cgc cta cgg cgc ctg ctg gcg cgg ccc ggg      711
Gly Glu Gln Asn Glu Glu Arg Leu Arg Arg Leu Leu Ala Arg Pro Gly
    155                 160                 165

ggt ggg cct ggc ggc gcg gcc cag cgc aag gtg cgc gcc ttc ttc cgc      759
Gly Gly Pro Gly Gly Ala Ala Gln Arg Lys Val Arg Ala Phe Phe Arg
170                 175                 180                 185

tcg tgc ctc gac atg cgc gag atc gag cga ctg ggc ccg cga ccc atg      807
Ser Cys Leu Asp Met Arg Glu Ile Glu Arg Leu Gly Pro Arg Pro Met
                190                 195                 200
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cta gag gtc atc gag gac tgc ggg ggc tgg gac ctg ggc ggc gcg gag      855
Leu Glu Val Ile Glu Asp Cys Gly Gly Trp Asp Leu Gly Gly Ala Glu
            205                 210                 215

gag cgt ccg ggg gtc gcg gcg cga tgg gac ctc aac cgg ctg ctg tac      903
Glu Arg Pro Gly Val Ala Ala Arg Trp Asp Leu Asn Arg Leu Leu Tyr
        220                 225                 230

aag gcg cag ggc gtg tac agc gcc gcc gcg ctc ttc tcg ctc acg gtc      951
Lys Ala Gln Gly Val Tyr Ser Ala Ala Ala Leu Phe Ser Leu Thr Val
    235                 240                 245

agc ctg gac gac agg aac tcc tcg cgc tac gtc atc cgc att gac cag      999
Ser Leu Asp Asp Arg Asn Ser Ser Arg Tyr Val Ile Arg Ile Asp Gln
250                 255                 260                 265

gat ggg ctc acc ctg cca gag agg acc ctg tac ctc gct cag gat gag     1047
Asp Gly Leu Thr Leu Pro Glu Arg Thr Leu Tyr Leu Ala Gln Asp Glu
                270                 275                 280

gac agt gag aag gtc ctg gca gca tac agg gtg ttc atg gag cga gtg     1095
Asp Ser Glu Lys Val Leu Ala Ala Tyr Arg Val Phe Met Glu Arg Val
            285                 290                 295

ctc agc ctc ctg ggt gca gac gct gtg gaa cag aag gcc caa gag atc     1143
Leu Ser Leu Leu Gly Ala Asp Ala Val Glu Gln Lys Ala Gln Glu Ile
        300                 305                 310

ctg caa gtg gag cag cag ctg gcc aac atc act gtg tca gag tat gac     1191
Leu Gln Val Glu Gln Gln Leu Ala Asn Ile Thr Val Ser Glu Tyr Asp
    315                 320                 325

gac cta cgg cga gat gtc agc tcc atg tac aac aag gtg acg ctg ggg     1239
Asp Leu Arg Arg Asp Val Ser Ser Met Tyr Asn Lys Val Thr Leu Gly
330                 335                 340                 345

cag ctg cag aag atc acc ccc cac ttg cgg tgg aag tgg ctg cta gac     1287
Gln Leu Gln Lys Ile Thr Pro His Leu Arg Trp Lys Trp Leu Leu Asp
                350                 355                 360

cag atc ttc cag gag gac ttc tca gag gaa gag gag gtg gtg ctg ctg     1335
Gln Ile Phe Gln Glu Asp Phe Ser Glu Glu Glu Glu Val Val Leu Leu
            365                 370                 375

gcg aca gac tac atg cag cag gtg tcg cag ctc atc cgc tcc aca ccc     1383
Ala Thr Asp Tyr Met Gln Gln Val Ser Gln Leu Ile Arg Ser Thr Pro
        380                 385                 390

cac cgg gtc ctg cac aac tac ctg gtg tgg cgc gtg gtg gtg gtc ctg     1431
His Arg Val Leu His Asn Tyr Leu Val Trp Arg Val Val Val Val Leu
    395                 400                 405

agt gaa cac ctg tcc ccg cca ttc cgt gag gca ctg cac gag ctg gca     1479
Ser Glu His Leu Ser Pro Pro Phe Arg Glu Ala Leu His Glu Leu Ala
410                 415                 420                 425

cag gag atg gag ggc agc gac aag cca cag gag ctg gcc cgg gtc tgc     1527
Gln Glu Met Glu Gly Ser Asp Lys Pro Gln Glu Leu Ala Arg Val Cys
                430                 435                 440

ttg ggc cag gcc aat cgc cac ttt ggc atg gcg ctt ggc gcc ctc ttt     1575
Leu Gly Gln Ala Asn Arg His Phe Gly Met Ala Leu Gly Ala Leu Phe
            445                 450                 455

gta cat gag cac ttc tca gct gcc agc aaa gcc aag gtg cag cag cta     1623
Val His Glu His Phe Ser Ala Ala Ser Lys Ala Lys Val Gln Gln Leu
        460                 465                 470

gtg gaa gac atc aag tac atc ctg ggc cag cgc ctg gag gag ctg gac     1671
Val Glu Asp Ile Lys Tyr Ile Leu Gly Gln Arg Leu Glu Glu Leu Asp
    475                 480                 485

tgg atg gac gcc gag acc agg gct gct gct cgg gcc aag ctc cag tac     1719
Trp Met Asp Ala Glu Thr Arg Ala Ala Ala Arg Ala Lys Leu Gln Tyr
490                 495                 500                 505

atg atg gtg atg gtc ggc tac ccg gac ttc ctg ctg aaa ccc gat gct     1767
Met Met Val Met Val Gly Tyr Pro Asp Phe Leu Leu Lys Pro Asp Ala
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                510                 515                 520

gtg gac aag gag tat gag ttt gag gtc cat gag aag acc tac ttc aag     1815
Val Asp Lys Glu Tyr Glu Phe Glu Val His Glu Lys Thr Tyr Phe Lys
            525                 530                 535

aac atc ttg aac agc atc cgc ttc agc atc cag ctc tca gtt aag aag     1863
Asn Ile Leu Asn Ser Ile Arg Phe Ser Ile Gln Leu Ser Val Lys Lys
        540                 545                 550

att cgg cag gag gtg gac aag tcc acg tgg ctg ctc ccc cca cag gcg     1911
Ile Arg Gln Glu Val Asp Lys Ser Thr Trp Leu Leu Pro Pro Gln Ala
    555                 560                 565

ctc aat gcc tac tat cta ccc aac aag aac cag atg gtg ttc ccc gcg     1959
Leu Asn Ala Tyr Tyr Leu Pro Asn Lys Asn Gln Met Val Phe Pro Ala
570                 575                 580                 585

ggc atc ctg cag ccc acc ctg tac gac cct gac ttc cca cag tct ctc     2007
Gly Ile Leu Gln Pro Thr Leu Tyr Asp Pro Asp Phe Pro Gln Ser Leu
                590                 595                 600

aac tac ggg ggc atc ggc acc atc att gga cat gag ctg acc cac ggc     2055
Asn Tyr Gly Gly Ile Gly Thr Ile Ile Gly His Glu Leu Thr His Gly
            605                 610                 615

tac gac gac tgg ggg ggc cag tat gac cgc tca ggg aac ctg ctg cac     2103
Tyr Asp Asp Trp Gly Gly Gln Tyr Asp Arg Ser Gly Asn Leu Leu His
        620                 625                 630

tgg tgg acg gag gcc tcc tac agc cgc ttc ctg cga aag gct gag tgc     2151
Trp Trp Thr Glu Ala Ser Tyr Ser Arg Phe Leu Arg Lys Ala Glu Cys
    635                 640                 645

atc gtc cgt ctc tat gac aac ttc act gtc tac aac cag cgg gtg aac     2199
Ile Val Arg Leu Tyr Asp Asn Phe Thr Val Tyr Asn Gln Arg Val Asn
650                 655                 660                 665

ggg aaa cac acg ctt ggg gag aac atc gca gat atg ggc ggc ctc aag     2247
Gly Lys His Thr Leu Gly Glu Asn Ile Ala Asp Met Gly Gly Leu Lys
                670                 675                 680

ctg gcc tac cac gcc tat cag aag tgg gtg cgg gag cac ggc cca gag     2295
Leu Ala Tyr His Ala Tyr Gln Lys Trp Val Arg Glu His Gly Pro Glu
            685                 690                 695

cac cca ctt ccc cgg ctc aag tac aca cat gac cag ctc ttc ttc att     2343
His Pro Leu Pro Arg Leu Lys Tyr Thr His Asp Gln Leu Phe Phe Ile
        700                 705                 710

gcc ttt gcc cag aac tgg tgc atc aag cgg cgg tcg cag tcc atc tac     2391
Ala Phe Ala Gln Asn Trp Cys Ile Lys Arg Arg Ser Gln Ser Ile Tyr
    715                 720                 725

ctg cag gtg ctg act gac aag cat gcc cct gag cac tac agg gtg ctg     2439
Leu Gln Val Leu Thr Asp Lys His Ala Pro Glu His Tyr Arg Val Leu
730                 735                 740                 745

ggc agt gtg tcc cag ttt gag gag ttt ggc cgg gtt tta cac tgt cca     2487
Gly Ser Val Ser Gln Phe Glu Glu Phe Gly Arg Val Leu His Cys Pro
                750                 755                 760

aag gtc tca ccc atg aac cct gcc cac aag tgt tcc gtg tgg             2529
Lys Val Ser Pro Met Asn Pro Ala His Lys Cys Ser Val Trp
            765                 770                 775

tgaccctggc tgcccgcctg cacgccccca ctgcccccgc acgaatcacc tcctgctggc   2589

taccggggca ggcatgcacc cggtgccagc cccgctctgg gcaccacctg ccttccagcc   2649

cctccaggac ccggtccccc tgctgcccct cacttcagga ggggcctgga gcagggtgag   2709

gctggacttt ggggggctgt gagggaaata tactggggtc cccagattct gctctaaggg   2769

ggccagaccc tctgccaggc tggattgtac gggccccacc ttcgctgtgt tcttgctgca   2829

agtctggtca aataaatcac tgcactgtta aaaaaaaaaa aa                      2871
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<210> SEQ ID NO 17
<211> LENGTH: 775
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 17

Met Glu Pro Pro Tyr Ser Leu Thr Ala His Tyr Asp Glu Phe Gln Glu
1 5 10 15

Val Lys Tyr Val Ser Arg Cys Gly Ala Gly Gly Ala Arg Gly Ala Ser
20 25 30

Leu Pro Pro Gly Phe Pro Leu Gly Ala Ala Arg Ser Ala Thr Gly Ala
35 40 45

Arg Ser Gly Leu Pro Arg Trp Asn Arg Arg Glu Val Cys Leu Leu Ser
50 55 60

Gly Leu Val Phe Ala Ala Gly Leu Cys Ala Ile Leu Ala Ala Met Leu
65 70 75 80

Ala Leu Lys Tyr Leu Gly Pro Val Ala Ala Gly Gly Gly Ala Cys Pro
85 90 95

Glu Gly Cys Pro Glu Arg Lys Ala Phe Ala Arg Ala Ala Arg Phe Leu
100 105 110

Ala Ala Asn Leu Asp Ala Ser Ile Asp Pro Cys Gln Asp Phe Tyr Ser
115 120 125

Phe Ala Cys Gly Gly Trp Leu Arg Arg His Ala Ile Pro Asp Asp Lys
130 135 140

Leu Thr Tyr Gly Thr Ile Ala Ala Ile Gly Glu Gln Asn Glu Glu Arg
145 150 155 160

Leu Arg Arg Leu Leu Ala Arg Pro Gly Gly Gly Pro Gly Gly Ala Ala
165 170 175

Gln Arg Lys Val Arg Ala Phe Phe Arg Ser Cys Leu Asp Met Arg Glu
180 185 190

Ile Glu Arg Leu Gly Pro Arg Pro Met Leu Glu Val Ile Glu Asp Cys
195 200 205

Gly Gly Trp Asp Leu Gly Gly Ala Glu Glu Arg Pro Gly Val Ala Ala
210 215 220

Arg Trp Asp Leu Asn Arg Leu Leu Tyr Lys Ala Gln Gly Val Tyr Ser
225 230 235 240

Ala Ala Ala Leu Phe Ser Leu Thr Val Ser Leu Asp Asp Arg Asn Ser
245 250 255

Ser Arg Tyr Val Ile Arg Ile Asp Gln Asp Gly Leu Thr Leu Pro Glu
260 265 270

Arg Thr Leu Tyr Leu Ala Gln Asp Glu Asp Ser Glu Lys Val Leu Ala
275 280 285

Ala Tyr Arg Val Phe Met Glu Arg Val Leu Ser Leu Leu Gly Ala Asp
290 295 300

Ala Val Glu Gln Lys Ala Gln Glu Ile Leu Gln Val Glu Gln Gln Leu
305 310 315 320

Ala Asn Ile Thr Val Ser Glu Tyr Asp Asp Leu Arg Arg Asp Val Ser
325 330 335

Ser Met Tyr Asn Lys Val Thr Leu Gly Gln Leu Gln Lys Ile Thr Pro
340 345 350

His Leu Arg Trp Lys Trp Leu Leu Asp Gln Ile Phe Gln Glu Asp Phe
355 360 365

Ser Glu Glu Glu Glu Val Val Leu Leu Ala Thr Asp Tyr Met Gln Gln
370 375 380

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Val Ser Gln Leu Ile Arg Ser Thr Pro His Arg Val Leu His Asn Tyr
385                 390                 395                 400

Leu Val Trp Arg Val Val Val Val Leu Ser Glu His Leu Ser Pro Pro
                405                 410                 415

Phe Arg Glu Ala Leu His Glu Leu Ala Gln Glu Met Glu Gly Ser Asp
            420                 425                 430

Lys Pro Gln Glu Leu Ala Arg Val Cys Leu Gly Gln Ala Asn Arg His
        435                 440                 445

Phe Gly Met Ala Leu Gly Ala Leu Phe Val His Glu His Phe Ser Ala
    450                 455                 460

Ala Ser Lys Ala Lys Val Gln Gln Leu Val Glu Asp Ile Lys Tyr Ile
465                 470                 475                 480

Leu Gly Gln Arg Leu Glu Glu Leu Asp Trp Met Asp Ala Glu Thr Arg
                485                 490                 495

Ala Ala Ala Arg Ala Lys Leu Gln Tyr Met Met Val Met Val Gly Tyr
            500                 505                 510

Pro Asp Phe Leu Leu Lys Pro Asp Ala Val Asp Lys Glu Tyr Glu Phe
        515                 520                 525

Glu Val His Glu Lys Thr Tyr Phe Lys Asn Ile Leu Asn Ser Ile Arg
    530                 535                 540

Phe Ser Ile Gln Leu Ser Val Lys Lys Ile Arg Gln Glu Val Asp Lys
545                 550                 555                 560

Ser Thr Trp Leu Leu Pro Pro Gln Ala Leu Asn Ala Tyr Tyr Leu Pro
                565                 570                 575

Asn Lys Asn Gln Met Val Phe Pro Ala Gly Ile Leu Gln Pro Thr Leu
            580                 585                 590

Tyr Asp Pro Asp Phe Pro Gln Ser Leu Asn Tyr Gly Gly Ile Gly Thr
        595                 600                 605

Ile Ile Gly His Glu Leu Thr His Gly Tyr Asp Asp Trp Gly Gly Gln
    610                 615                 620

Tyr Asp Arg Ser Gly Asn Leu Leu His Trp Trp Thr Glu Ala Ser Tyr
625                 630                 635                 640

Ser Arg Phe Leu Arg Lys Ala Glu Cys Ile Val Arg Leu Tyr Asp Asn
                645                 650                 655

Phe Thr Val Tyr Asn Gln Arg Val Asn Gly Lys His Thr Leu Gly Glu
            660                 665                 670

Asn Ile Ala Asp Met Gly Gly Leu Lys Leu Ala Tyr His Ala Tyr Gln
        675                 680                 685

Lys Trp Val Arg Glu His Gly Pro Glu His Pro Leu Pro Arg Leu Lys
    690                 695                 700

Tyr Thr His Asp Gln Leu Phe Phe Ile Ala Phe Ala Gln Asn Trp Cys
705                 710                 715                 720

Ile Lys Arg Arg Ser Gln Ser Ile Tyr Leu Gln Val Leu Thr Asp Lys
                725                 730                 735

His Ala Pro Glu His Tyr Arg Val Leu Gly Ser Val Ser Gln Phe Glu
            740                 745                 750

Glu Phe Gly Arg Val Leu His Cys Pro Lys Val Ser Pro Met Asn Pro
        755                 760                 765

Ala His Lys Cys Ser Val Trp
    770                 775
```

What is claimed is:

1. An isolated nucleic acid comprising a nucleotide sequence encoding a metallopeptidase having at least about 95% amino acid sequence identity with the complete amino acid sequence of SEQ ID NO: 13.

2. A recombinant vector comprising the isolated nucleotide sequence of claim **1**.

3. An isolated host cell transformed with the vector of claim **2**.

4. An isolated nucleic acid comprising the nucleotide sequence of SEQ ID NO: 12.

5. A recombinant vector comprising the isolated nucleotide sequence of claim **4**.

6. An isolated host cell transformed with the vector of claim **5**.

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